Distribution and Stability of Soil Carbon in Spekboom Thicket, Eastern Cape, South Africa

By

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DECLARATION

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ABSTRACT

Soils of semi-arid regions generally have low organic matter contents, primarily attributed to prevailing climatic conditions. Yet, the spekboom thicket, located in the semi-arid region of the Eastern Cape (South Africa) has been distinguished to accumulate large amounts of soil carbon. To further understand this remarkable accumulation, a detailed investigation was carried out to evaluate the distribution, speciation and stability of the soil carbon from directly beneath spekboom (*Portulacaria afra*) and other "nonspekboom" vegetation within the intact thicket, and from adjacent, degraded (overgrazed), open thicket sites.

In the first experimental chapter, the distribution of organic and inorganic C, as well as general soil properties (pH, EC, exchangeable and water soluble cations, hydrophobicity, water stable aggregates) were investigated in the intact (spekboom and nonspekboom) and degraded (open) thicket soils. There were no significant differences in the C content (organic or inorganic) between the spekboom and nonspekboom sites in the intact thicket. Soil organic carbon (SOC) content however was greatly influenced by thicket degradation, whereas, inorganic C remained unaffected. Mean organic C (0-50 cm) ranged from 19-34, 22-62, and 19-58 g C kg^{-1} for open, spekboom, and nonspekboom sites, respectively. Whereas, mean inorganic C ranged from 2-9 g C kg^{-1} irrespective of sites, representing 4-22 % of total soil C. However, no significant differences were detected between degraded and intact sites for all (total, organic and inorganic) C stocks, attributed to variation in bulk density.

Spekboom soils contained significantly higher concentrations of exchangeable and water soluble Mg, Na, and K, and consequently had higher pH and EC compared to the other sites. Soils tended to be hydrophobic especially at shallower depths in the intact thicket irrespective of vegetation type, whereas soils from the adjacent open sites were not significantly hydrophobic. Macro-aggregates were considerably more stable under intact sites compared to open sites.

The second experimental chapter investigated the effects of degradation and vegetation type on the stability and structural chemistry of SOC. This involved partitioning the soil organic matter into particulate and mineral-bound (stable) fractions, and then examining the relationships between the stable organic C fraction and various soil properties as an attempt to elucidate the stabilization mechanism(s). Particulate organic C ranged from 7.0, 9.3, and 14.4 g C kg^{-1} for open, spekboom, and nonspekboom respectively; representing 22-34 % of total SOC. Stable (mineral-bound) organic C accounted for the largest fraction *ca*. 60-66 % of total SOC; 28.0 and 26.2 g C kg^{-1} for spekboom and non-spekboom respectively, versus 17.2 g C kg^{-1} for open sites. It was concluded that SOC stability was influenced by the inherent residue quality (recalcitrance) rather than soil properties, attributed to the aliphatic (lipids and waxes) and highly aromatic nature of the spekboom litter substrate and particulate OM. These research findings are of fundamental significance in understanding soil organic matter stabilization in semi-arid environments.

UITTREKSEL

Gronde van semi–ariede streke het oor die algemeen 'n lae organiese materiaalinhoud, wat hoofsaaklik aan die heersende klimaatstoestande toegeskryf kan word. Tog, het die spekboomruigtes, geleë in die semi-ariede streek van die Oos-Kaap (Suid Afrika), onderskeidelik groot hoeveelhede grondkoolstof geakkumuleer. Om hierdie merkwaardige akkumulasie verder te verstaan, was 'n gedetailleerde ondersoek uitgevoer om die verspreiding, spesiasie en stabiliteit van die grondkoolstof direk onder spekboom (*Portulacaria afra*) en ander “nie-spekboom” plantegroei binne die intakte ruigte en van die aangrensende gedegradeer (oorbeweide), oop gras areas te evalueer.

In die eerste eksperimentele hoofstuk, is die verspreiding van organiese en anorganiese C, sowel as algemene grondeienskappe (pH, uitruilbare en wateroplosbare katione, hidrofobisiteit, water-stabiele aggregate) was in die intakte (spekboom en nie-spekboom) en degradeerde (oop) ruigtes ondersoek. Daar was geen betekenisvolle verskille in die C-inhoud (organies of anorganies) tussen die spekboom en nie-spekboom gronde in die intakte ruigtes gevind nie. Grond organiese koolstof (GOK) inhoud is grootliks deur die ruigte se agteruitgang beïnvloed, maar anorganiese C-voorrade was egter nie beïnvloed nie. Die gemiddelde organiese C-inhoud (0-50 cm) het van 19-34, 22-62 en 19-58 g C kg^{-1} onderskeidelik vir 'n oop, spekboom, en nie-spekboom areas gewissel. Die anorganiese C-inhoude het vanaf 2-9 g C kg^{-1} gewissel, ongeag van ligging, wat 4-22 % van die totale grond C uit maak. Daar is egter geen beduidende verskille tussen vervalle en heel terreine vir almal (totaal, organiese en anorganiese) C aandele opgespoor, toegeskryf word aan die variasie in die massadigtheid.

Spekboom gronde het beduidende hoër konsentrasies van uitruilbare en wateroplosbare Mg, Na, en K waardes bevat. Dit het ook hoër pH en EC, in vergelyking met die ander areas gehad. Gronde was geneig om hidrofobies te wees, veral op vlakker dieptes in die intakte ruigte, ongeag van die plantegroei, terwyl die gronde van die aangrensende oop areas nie beduidend hidrofobies was nie. Makro grond aggregate was aansienlik meer stabiel onder intakte ruigtes as in die oop areas.

Die tweede eksperimentele hoofstuk het die gevolge van die weiding-geïnduseerde degradasie en tipe plantegroei op die stabiliteit en strukturele chemie van GOK ondersoek. Dit het die skeiding van die grond organiese materiaal in die vrye en mineraalgebonde (stabiele) fraksies behels. Daarna is die verhoudings tussen die stabiele organiese C fraksie en verskeie grondeienskappe ondersoek om die stabiliseringmeganisme(s) uit te lig. Die chemiese aard van die blare, plante oorblyfsels, fyn en minerale gebinde organiese C is met behulp van spektroskopie ondersoek. Vrye organiese C voorraad het van 7.0, 9.3, en 14.4 g C kg^{-1} vir oop, spekboom, en nie-spekboom onderskeidelik gewissel, wat ongeveer 23-34% van die totale GOK verteenwoordig. Stabiele (minerale gebind) organiese C-voorrade is vir die grootste fraksie ca. 60-66% van die totale GOK verantwoordelik. Dit was 28.0 en 26.2 g C kg^{-1} onderskeidelik vir spekboom en nie-spekboom, teenoor 17.2 g C kg^{-1} vir 'n oop area. Die mineraalgebonde GOK het geen noemenswaardige korrelasies met die ondersoekte grondeienskappe getoon nie. Dus is tot die gevolgtrekking gekom dat GOK stabilisering eerder deur die plante oorblyfsel se chemiese kwaliteit beïnvloed word. Strukturele chemiese karakterisering (^{13}C CP-MAS KMR) van die spekboom afval en vrye C fraksie het gewys dat weerspannigheid die mees prominente meganisme van koolstof stabilisering van organiese koolstof in die gronde is. Dit word aan die alifatiese (lipiede en wasse) en sterk aromatiese aard van die spekboom substrate toegeskryf. Hierdie ondersoek het tot die fundamentele kennis van grond organiese materiaalstabilisering in 'n semi-ariede omgewings bygedra.

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GENERAL INTRODUCTION AND RESEARCH AIMS

Carbon dioxide (CO_2) is one of the potent greenhouse gases (GHGs). Soils constitute the largest terrestrial carbon (C) reservoir and have a potential to serve as either a source or a sink for CO_2 (Lal, 2003, 2004 and Schlesinger, 2000). Carbon sequestration is a strategic approach to reduce CO_2 accumulation in the atmosphere through plant photosynthesis and precipitation of C as secondary carbonates in soil (Lal, 1998). This requires a thorough understanding of the soil properties and processes that affect soil carbon, both the “organic” and “inorganic” components. Most of the previous research on soil organic matter stabilization has been focused on temperate climates (von Lutzow et al., 2006).

The unique succulent spekboom thicket is located in the semi-arid region of the Eastern Cape Province, South Africa. Previous research e.g. Lechmere-Oertel (2003), Lechmere-Oertel et al. (2008), Mills et al. (2005), and Mills and Fey (2004) has revealed the remarkable soil C accumulation in the spekboom thicket. However, a substantial portion of this naturally dense thicket has been extensively degraded into a savanna-type ecosystem by over-grazing. Mills and Fey (2004) found the degradation to cause a significant reduction in total C content (30 vs. 56 g C kg^{-1} for degraded and intact thicket sites, respectively). However, it remains unknown to what extent this total C consists of organic or inorganic C. Several soil C studies conducted in semi-arid ecosystems, e.g. Gallardo and Merino (1993), Martínez et al. (2007), White et al. (2009), and Rasmussen and White (2010), have demonstrated the important influence of vegetation type on soil organic carbon (SOC) content. It remains to be determined whether the remarkable SOC accumulation in spekboom thickets is explicitly due to the spekboom plant (*Portulacaria afra*). The soil chemical and physical properties beneath spekboom and nonspekboom vegetation in intact thicket have also never been explored. The extent of hydrophobicity and aggregate stability of intact and degraded thicket soils has never been studied. Furthermore, the carbon stability and stabilization mechanisms of SOC in thicket soils have never been explored before.

Therefore, the first objective of this study was to quantify organic and inorganic C stocks, and examine soil properties at degraded open, and intact spekboom, and nonspekboom sites. The second objective of this study was to examine the stability of SOC in intact and degraded thicket, and subsequently to elucidate the stabilization mechanism(s) involved by investigating the relationships between the stable SOC fraction and selected soil properties (clay content, aggregate stability, hydrophobicity, mineralogy and polyvalent cations) and by examining the structural chemistry of the SOC.

CHAPTER ONE: A LITERATURE REVIEW

1.1 CARBON STABILIZATION IN SOILS

Several carbon stabilization mechanisms have been proposed and reviewed by various authors e.g. Christensen (1996), Sollins et al. (1996), von Lützow et al. (2006), relating to the protection and stabilization of the soil organic matter (OM) against microbial decomposition and other losses. These mechanisms can be broadly categorized into three groups; (i) biochemical recalcitrance of the OM substrate, (ii) physical protection, which renders SOM spatially inaccessible for microbial decomposition, and (iii) chemical stabilization due to interactions between the SOM and mineral components (Palumbo et al., 2004). More than one of these mechanisms may operate simultaneously to various degrees in soil or even within an individual soil horizon (von Lützow et al., 2006). The extent to which carbon accumulates and is sequestered in soil is largely determined by the net primary production input i.e. its quantity and quality, and its decomposition rate (von Lützow et al., 2006).

SOM decomposition can be biotic (microorganisms and enzymes) or abiotic (chemical oxidation), and can generally be categorized into three distinct phases as suggested by Jenkinson and Ladd (1981), with each phase comprising of varying functional pools and characteristic turnover rates. The first phase of decomposition exhibits a rapid decomposition rate and a quick turnover time, typically less than 10 years, this type of OM pool is commonly known as the active or labile OM. The second phase is characterized by slower decomposition rate and a relatively long turnover time, decades in temperate climates, the OM pool encountered in this phase is termed the intermediate OM. At complete decay, the third phase represents the refractory, also known as a passive pool at a very slow decomposition rate and long turnover time, centuries to millennia in temperate climates (Lavelle et al., 1993, von Lützow et al., 2006, Jenkinson and Ladd, 1981, Marín-Spiotta et al., 2008).

1.1.1 Biochemical recalcitrance

Organic matter can be stabilized in soil against microbial decomposition or degradation due to its inherent recalcitrance, this relates to the chemical characteristics intrinsic to the OM substrate (Mikutta et al., 2006, Palumbo et al., 2004). The concept of organic matter recalcitrance encompasses the molecular-level characteristics of organic substances, including their elemental composition, presence of functional groups, as well as molecular conformation, which in turn influences their degree of degradation by microbes and enzymes (Sollins et al., 1996). Von Lützow et al. (2006) distinguished between primary and secondary recalcitrance, the former pertains to the recalcitrance of plant litter and rhizo-deposits as a function of their inherent indigenous molecular characteristics, whereas the latter relates to the transformed humified nature of the organic matter.

1.1.1.1 Primary recalcitrance

Soil organic matter consists of humic and non-humic substances, the latter has recognizable physical and chemical properties, and consists mainly of carbohydrates, proteins, peptides, amino acids, fats, waxes and low molecular weight acids (Derenne and Largeau, 2001, von Lützow et al., 2006), and thus contributes to the primary recalcitrance. Plant residues, above and below ground (leaves and roots) comprises of a range of complex mixtures of organic components; mostly polysaccharides and lignin, as well as proteins, polyphenols, chlorophyll, cutin, suberin, lipids and waxes (von Lützow et al., 2006). Some of these e.g. lignin, waxes, cutin, and suberin are considered to be relatively resistant to degradation due to their molecular conformation and structural composition (Derenne and Largeau, 2001, Kögel-Knabner, 2002, Palumbo et al., 2004, von Lützow et al., 2006). Kögel-Knabner (2002) reported the variation in relative abundance of these components between plant species. Findings from previous studies in Sonoran desert ecosystems claim litter quality, particularly lignin content to have a significant control over leaf litter decomposition rate (Martínez et al., 2007). Some authors e.g. Kalbitz et al. (2003a, 2003b) have reported these components as rather selectively preserved during the initial phase of degradation of plant residues. However, the conception of selective preservation remains disputable as recent studies with CPMAS ^{13}C NMR and pyrolysis techniques have confirmed lignin to be altered quite

readily, and does not seem to be stabilized in the long term in any soil fraction (Baldock and Nelson, 2000, Kiem and Kögel-Knabner, 2003, Kögel-Knabner, 2000).

1.1.1.2 Secondary recalcitrance

Secondary recalcitrance refers to selective degradation of microbial products, humic polymers and charred material (Aiken et al., 1985b; Sollins et al., 1996; von Lützow et al., 2006). Soil fauna and microbes tend to selectively degrade the less recalcitrant compounds, which leaves the refractory portion of the residue that is resistant to further degradation, and thus increase the average recalcitrance of the residue (Sollins et al., 1996).

1.1.2 Spatial inaccessibility

Accessibility of the organic matter substrate to microorganisms is the prime requirement for OM decomposition to take place; freshly deposited particulate organic matter serves as a substrate for microbial decomposition. Exclusion from water, nutrients, and oxygen of the OM substrate tends to reduce degradability by microorganisms due to limited microbial activity under such conditions (Bachmann et al., 2008, Baldock and Nelson, 2000). Furthermore, the spatial location of organic substances influences their accessibility to microbes and enzymes. Factors such as hydrophobic nature of the OM, occlusion within soil aggregates, intercalation into expandable (2:1) clays, and encapsulation by organic macromolecules contribute to spatial inaccessibility of OM (von Lützow et al., 2006).

1.1.2.1 Soil aggregation

Physical stabilization of OM through formation of soil aggregates relates to the accumulation of organic matter as a result of establishment of physical barriers between microbes and their enzymes and the OM substrate (Jastrow et al., 1996, Six et al., 2004). Organic matter spatially protected by occlusion within aggregates is shielded against decomposition and stabilized due to restricted room for microbial activity and restricted aerobic decomposition due to limited diffusion of O_2 and extracellular enzymes (von Lützow et al., 2006). Pore size distribution plays a major role in this regard because of its influence on the proportion of pores filled with water to those available and capable to host microorganisms (Hillel, 1980), also restricts

accessibility of faunal predators (von Lützow et al., 2006). Doran and Smith (1980, 1987) and Elliott and Coleman (1988) reported that exclusion of faunal predators raises the energy efficiency of microorganisms and induces a slower microbial turnover in soils with a finer pore system. Christensen (1992) discerned that physical fractionation methods strictly involve density and size separation, without considering aggregates. Balabane and Plate (2004) quantified a distinction between primary particles and equally sized aggregates in the silt size fraction, and further suggested that such entities are likely comprised of different capabilities to stabilize SOM. Tisdall and Oades (1982) found microaggregates (53 – 250 µm) to be more stable and less susceptible to disruption than macroaggregates (250 – 2000 µm). Aggregates < 20 µm are supposedly very stable and only disrupted by ultrasonic dispersion (Oades & Waters, 1991), whereas aggregates < 2 µm even survive ultrasonic treatment (Chenu and Plante, 2006).

1.1.2.2 Intercalation

Protection by means of intercalation of the organic molecules into the interlayer voids of expandable clay minerals was proposed by Piccolo (2002) based on the conventional conceptions of humification, that humic substances are formed through partial decomposition of original polymers of plant residues (Martin and Haider, 1971, Schnitzer and Khan, 1972, Stevenson, 1994, Waksman, 1938). According to these conceptions, humic substances have unique chemical structures that differ from their precursor plant polymers and are resistant to degradation by normal microbial enzymes (Hedges, 1988, Stevenson, 1994) due to their recalcitrance. Knicker et al. (1996) and Zang et al. (2000) suggested that labile OM is protected from degradation via intercalation and subsequent encapsulation by the network of recalcitrant humic pseudo-macromolecules. This is in agreement with suggestions of Piccolo (2000) and Spaccini et al. (2002) concerning hydrophobic protection of labile molecules by hydrophobic domains of humic pseudo-macromolecules. However, evidence in natural soil conditions is limited for this intercalation and encapsulation phenomenon of labile OM in organic pseudo-macromolecules (von Lützow et al., 2006), direct measurements *in situ* and empirical data regarding this concept as a mechanism of OM protection are necessary to fill the gaps in knowledge for future research.

1.1.2.3 Encapsulation

The conceptions of molecular aggregation and supramolecular structure (Piccolo, 1996) describes humic substances (HS) as high molecular weight polymers, comprised of small heterogeneous molecules of amphiphilic nature. According to Piccolo (2002) and his conceptual model of humification, humic substances can be viewed as supra-molecular associations of small, heterogeneous, and self-assembling “clinging” molecules, derived from the decomposition of plant and microbial residues. Such associations tend to form clusters by hydrophobic interactions and hydrogen bonding, hence large characteristic molecular “pseudo-macromolecular” size (Piccolo, 2002, von Lützow et al., 2006).
The amphiphilic nature of humic substances simply implies possession of differing polarities within one molecule (Schaumann, 2006), this unique feature enables HS to rearrange themselves to form molecular aggregates or supra-molecular associations in solution e.g. formation of micelles and bi-layer membranes on mineral surfaces. The rearrangement occurs in such a manner that in solution; the hydrophobic domains of the amphiphilic molecules align themselves to form the interior of molecular aggregates, or a comparable bi-layer on mineral surfaces, whilst the polar “hydrophilic” carboxylic groups constitute the exterior surfaces (Wershaw, 1986). Such humic associations seem to interact very closely with the fresh organic matter entering the soil as litter or plant residues (Schaumann, 2006). Furthermore, Schnitzer and Khan (1972) proposed that fluvic acid (FA) molecules in aqueous solutions are linked together by hydrogen bonds between phenolic and benzene-carboxylic acid groups to form loose aggregates with structural characteristics described as punctured by voids of varying dimensions that can trap and fix organic molecules.

The concept of OM protection via encapsulation within organic macromolecules relates to hydrophobic protection provided by non-polar domain of humic substances (von Lützow et al., 2006, Piccolo, 2002). Piccolo (1996) proposed that humified organic carbon (OC), particularly humic acids (HA) and humin, represents the most persistent pool of OC in soil, with mean resident time of several centuries, which suggest that protection and relative stability of OM encapsulated in the hydrophobic interior domains of aggregate structures of such molecules will be enhanced, with longer mean residence time. Despite the lack of substantial evidence for this

phenomenon under natural soil conditions, however, interior hydrophobic domains are likely to form under natural organic matter in soil, and are considered environmentally valuable for its ability to serve as a sorbent for hydrophobic organic chemicals (Schaumann, 2006).

1.1.2.4 Hydrophobicity

Water presides over microbial mobility, nutrient and enzyme distribution, as well as O_2 availability with reference to soil microbial processes (Bachmann et al., 2008). Hydrophobicity “water exclusion” of aggregates reduces surface wettability and thus accessibility and interaction of the organic matter with microorganisms (Goebel et al., 2004; Jandl et al., 2004); this enhances aggregate stability (Piccolo & Mbagwu, 1999) and further contributes to intra-aggregate occlusion of OM (Goebel et al., 2005). This phenomenon is even more prominent during dry spells, typically following rainfall events especially in semi-arid and arid regions (Hillel, 1980). The significance of the relationship between aggregate stability and the destructive action of water is well emphasized by Hillel (1980) on his suggestion that the bonding substances dissolve and weaken as the clay swells and possibly disperses, this is manifested by the tendency of aggregates to collapse and disintegrate upon wetting (hydration). In addition to this, he highlighted the impact of raindrops and flowing water, which tends to detach surface soil particles and transport them away as suspended material in runoff water during erosion, consequential abrasion by transported particles scours the surface and contributes to disintegration of the soil structure. Goebel et al. (2005) found supporting evidence for great stability of hydrophobic OM and enhanced stability of intra-aggregate (occluded) OM due to hydrophobic aggregate surfaces.

1.1.3 Interactions with the mineral phase

Chemical stabilization of the soil organic matter involves intermolecular interactions between organic and inorganic substances that reduce the availability of the organic substrate due to complexation of functional groups and change in conformation (Guggenberger and Kaiser, 2003). Various mechanisms have been identified according to which the organic matter can interact with the mineral phase in soil, such as ligand exchange, polyvalent cation bridges, and weak interactions such as

van der Waals forces and H-bonding (Theng, 1979, Oades, 1989). Mikutta et al. (2007) concluded that stability and consequent resistance of sorbed OM to biodegradation increases as more mechanisms are involved simultaneously during the attachment of OM to mineral surfaces.

Tisdall and Oades (1982) proposed the formation and stabilization of aggregates to be hierarchical (<0.2 µm, 0.2-2 µm, 2-20 µm, 20-250 µm, and >2000 µm); that primary particles are bound together to form aggregates through the action of soil biota, where microbial secretions, root exudates, and faunal mucus serve as binding agents. Furthermore, they suggested that aggregates can also be bound together with fungal and plant debris into even larger aggregates. OM occlusion in this manner is operative over long time scales and is assisted by pedogenic processes such as clay mineral weathering, and formation of Fe- and Al- oxides and hydroxides (von Lützow et al., 2006, Mayer et al., 2004). The relative importance of mineral sorption for long-term OM stabilization in the field has been affirmed by slower decomposition rates of OM associated with mineral surfaces from incubation experiments, as well as a positive correlation obtained between the organic carbon content and mineral phase properties (Miltner and Zech, 1998, Jones and Edwards, 1998, van Hees et al., 2003, Kalbitz et al., 2005). Mineral surfaces of Fe and Al oxides and hydroxides are major sorbents of dissolved organic matter in most soils (Kaiser and Guggenberger, 2007), evidence was provided by Singer and Huang (1993) on their observation that humic, tannic, and citric acid associated with non-crystalline Al hydroxide were only partly destructible by H_2O_2 and NaOCl, while tannic and citric acid were completely oxidized in the absence of Al hydroxide. Such behavior of high resistance to destruction of mineral-associated organic compounds can be explained by co-precipitation or formation of strong surface complexes at the mineral surface (Mikutta et al., 2005). Several mechanisms have been proposed (Oades, 1989, Theng, 1979, Vermeer et al., 1998) for OM interactions with the inorganic (minerals and metal oxides) components in soils, such as ligand exchange, polyvalent cation bridging, and weak interactions involving Van der Waals forces.

1.1.3.1 Ligand exchange

Ligand exchange relates to the displacement of hydroxyl groups (deprotonation) by organic functional groups on simple coordinated OH groups on mineral surfaces, as well as carboxyl and phenolic OH groups of the OM as a mechanism for the formation of stable organio-mineral associations in soils (Mikutta et al., 2007, Gu et al., 1994). Furthermore, complexation of OM via ligand exchange increases with decreasing pH (Gu et al., 1994). Gu et al. (1994) suggested that the requirement of large amount of heat for adsorption and corresponding little desorbability of sorbed OM on Fe oxides via ligand exchange reactions was likely due to strong chemical bonds established between the organic molecules and the mineral surface (Jones and Edwards, 1998, Kalbitz et al., 2005), which reduced degradability of sorbed OM.

1.1.3.2 Polyvalent cation bridging

Polyvalent cations play a major role in the retention of OM on both organic (e.g. OM itself) and inorganic (e.g. clay minerals) colloids. Polyvalent cations tend to bridge the negatively charged clay particles together, and organic macromolecules to each other, additionally, since both clays and OM are negatively charged, they are also bridged together in the presence of polyvalent cations (Oades, 1988). Polyvalent cations are well known for their ability to maintain neutrality at the mineral surface by neutralizing the charge on both the mineral surface and the acidic functional group of the OM, thus acts as a bridge between two, sometimes even more charged sites, whilst retaining the OM sorbed on the surface of minerals (von Lützow et al., 2006). The most predominant polyvalent cations in neutral and alkaline soil environments are Ca^{2+} and Mg^{2+}, with hydroxyl-polycations of Fe^{3+} and Al^{3+} most prevalent in acid soil conditions (von Lützow et al., 2006).

The relevance of cation bridging as a mechanism of OM protection against microbial degradation relates to the complexation of carboxyl groups of organic molecules with multivalent cations bound on mineral surfaces via mineral-OM interaction, or complexation of carboxyl groups amongst organic molecules on their own i.e. OM-OM interaction (Mikutta et al., 2007, Oades, 1988). Although the bonding efficiency of OM via formation of electrostatic cation bridges is generally weaker than that of ligand exchange (Benke et al., 1999, Kaiser and Zech, 2000), cation bridging becomes more relevant to the stability of the soil microstructure at neutral to alkaline

pH conditions (von Lützow et al., 2006), where it induces flocculation and subsequent precipitation of metal-OM complexes e.g. Ca-OM (Oades, 1988). In addition to this, Oades (1988) stated that surfaces of both clay minerals and OM are least accessible to microorganisms in their flocculated state, and that the large OM content of calcareous soils is attributed to the Ca^{2+} bridging effect. This phenomenon is witnessed by restricted swelling of expandible clays in the presence of polyvalent cations (Oades, 1988). Post et al. (2004) reported that the presence of polyvalent cations on the mineral exchange complex, especially calcium (Ca^{2+}), facilitates sorption of organic polymers to soil minerals via cation bridging of OM to permanently negatively charged siloxane surfaces, and hydroxyls of phyllosilicates and oxides (Mikutta et al., 2007), so as to protect the organic compounds against further microbial and chemical attack. Mikutta et al. (2007) provided evidence through his sorption experiments, where he examined OM sorption to minerals using equimolar concentrations of $CaCl_2$, NaCl, and NaCl-NaH_2PO_4 as background electrolytes. The notion behind his empirical approach was that the presence of $CaCl_2$ would enhance sorption of OM via Ca^{2+} bridging. His results displayed larger sorption in $CaCl_2$ than in other background solutions, which suggests formation of cation (Ca^{2+}) and water bridges at the mineral surfaces. However, the effect of Ca^{2+} on OM sorption was found to be negligible at low pH, and contrastingly more prominent at neutral pH. This corresponds with the results of Varadachari et al. (2000) and Weng et al. (2005), that Ca^{2+} increases sorption of humic and fluvic acid (organic compounds) to goethite at neutral to alkaline pH as compared to NaCl systems. Although anions are normally repelled from negatively charged mineral surfaces, binding does occur in the presence of polyvalent cations on the exchange complex (von Lützow et al., 2006).

1.1.3.3 Weak interactions

Wershaw (1999) suggested that clay minerals and metal oxides provide surfaces upon which amphiphilic humic moieties form bi-layer, membrane-like coatings. Amphiphilic molecules exhibit surface-active properties (Wershaw, 1999), upon which weak intermolecular interactions such as hydrophobic interactions and hydrogen bonding may occur, they tend to orient themselves to form bi-layer membrane-like coatings on mineral and hydrous oxide surfaces, such that the non-polar parts of the molecules constitute the interior of the membrane and the polar

carboxylic acid groups constituting the exterior surfaces of the membrane (Schaumann, 2006). Hydrophobic interactions occur via Van der Waals forces, driven by exclusion of hydrophobic residues from water, whereas hydrogen bonds on the other hand are formed partially via interaction of hydrogen atoms with positive partial charge, with partially negatively charged other (O or N) atoms (von Lützow et al., 2006). Weak interactions have also been encountered in special instances where polar (hydrophilic) groups occur in the interiors of aggregates, under such situations, the affinity for water of polar groups is reduced by bonding to other polar groups via weak interactions e.g. hydrogen bonding (Fendler, 1984).

1.2 CARBON STABILIZATION MECHANISMS IN SEMI-ARID SOILS

Semi-arid savannas are largely dynamic and often involve more than one steady state (Rietkerk et al., 1996). Disturbances such as grazing and bush fires typically encourage transition from one state to the next (Van de Koppel and Prins, 1998). Heavy grazing encourages competitive relationships among the different vegetation species, such that the heavily grazed (often the palatable) species loose competitive power over the lightly grazed ones. In most African savannas, intensified grazing has greatly reduced plant species diversity in this manner (Sarmiento, 1992); grazing intolerant species are defoliated and eventually disappear, followed by subsequent replacement by less palatable annual species (Tessema et at., 2011).

A substantial amount of research has been carried out on elucidating soil C stabilization mechanisms and its dynamics in temperate and tropical regions. However, semi-arid soils differ quite significantly from temperate soils in mineralogical and chemical composition, and rates of climate dependent chemical processes such as weathering and biodegradation. Mechanisms of carbon stabilization in semi-arid soils deserve attention, as little is known about its role and contribution to the carbon cycle.

Generally, semi-arid regions are characterized by high evaporation and low annual precipitation; as a norm, soils from such regions are commonly known to contain low quantities of organic carbon in the order of 35-42 ton C ha^{-1} (White et al., 2009). This is primarily attributed to prevailing climatic regimes under such environments, which favor the decomposition of organic residues (Davidson and Janssens, 2006;

Stevenson, 1994). However, in contrast to a common trend often experienced in ecosystems, that biomass production increases along a rainfall gradient from deserts to forests; a remarkable exception is observed under the succulent spekboom thicket, where an average of about 5 % organic C was noted in the topsoil. Mills et al. (2005) estimated about 450 g m^{-2} $year^{-1}$ leaf litter production by spekboom, which is exceptionally high for a semi-arid region and resembles that of mesic forest ecosystems with higher rainfall regimes. Litter quality and its resistance to degradation is one of the possible explanations for such unusual accumulation of soil carbon owing to the waxy, non-combustible nature of the spekboom, which is the dominant shrub in the thicket. White et al. (2009) suggested that litter dynamics, both chemistry and quality varies with vegetation type, and are most likely to control the rates of litter decomposition and SOC accumulation in arid ecosystems (White et al., 2009). Furthermore, he suggested that variation in SOC by vegetation may be attributed to the differences in SOC recalcitrance and soil microbial communities among vegetation types. This was confirmed by a good correlation observed between vegetation type and rates of litter decomposition in semi-arid ecosystem studies carried out by Gallardo and Merino (1993) and Vaieretti et al. (2005). Variation in temperature and precipitation constitute the foremost controlling factors for soil organic carbon (SOC) cycling over regional to global scale (Jenny, 1980, Post et al., 1982), in addition to this, White et al., (2009) suggested the timing and frequency of precipitation to be the overriding factors of climatic control for SOC cycling in arid and semi-arid ecosystems.

The extensive, closed-canopy foliage of spekboom plays a significant role in shading the soil surface, thereby reducing the temperature effects on SOM mineralization; moreover, this favours formation of stable aggregates through interception of rainfall (Mills and Fey 2004). The reduced rate of OM mineralization in the thicket may possibly be explained through the altered soil microclimate from the resultant cooling and reduction in frequent wetting of the soil surface from shading and interception of rainfall (Birch 1958; Jenkinson 1981; Thurow et al. 1987; Mills et al., 2005). Hillel (1980) suggested that processes associated with drying tend to promote aggregate stabilization by allowing glue-like organic gums and gels to coagulate and set irreversibly to serve as stable cementing agents.

Approximately 35 to 40 % of the global land area constitutes arid semi-arid regions (White et al., 2009). However, over the past several years, most interest has been directed on acid-neutral pH soils, and temperate to tropical regions of the world. As a result, an appreciable deficiency in knowledge and understanding concerning the OM stabilization mechanisms operating in semi-arid to arid regions and alkaline (pH > 7) soils, as well as the relative function of these soils in the global C cycle remains obscure. The present study serves to review existing scientific literature and fill the vacant gaps in knowledge pertaining to the significance of the different soil organic matter pools as well as the mechanisms responsible for their stabilization in semi-arid soils, and to ascertain if organic matter accumulation in these soils is attributable to the type of vegetation.

Carbon sequestration as well as the capacity of the soil to serve as a sink for atmospheric CO_2 is largely dependent on the rate of soil organic matter mineralization. Thus, better understanding of the mechanisms that stabilize organic matter in soils and the exceptional ability of the dynamic humic substances to retard the rate of mineralization of the sensitive labile soil organic matter pool is indispensable for the preservation of ecological atmospheric equilibrium (Piccolo, 1996).

1.3 RESEARCH METHODOLOGIES

Soil organic matter consists of various functional pools, defined by their characteristic turnover times and pool sizes (von Lützow et al., 2007, Jenkinson and Ladd, 1981). Different specific stabilization mechanisms are responsible for stabilization of SOM pools, as illustrated briefly in Table 1.1 below (page 17); such pools explicitly represent unique functional pools (von Lützow et al., 2007).

1.3.1 Isolation of SOM fractions

A range of fractionation methods have been recommended by various authors regarding isolation of different SOM fractional pools. A suitable fractionation method must be able to identify functionally different but unique SOM pools with a uniform (homogeneous) decay behavior in terms of turnover rates (Bruun et al., 2004, Smith et al., 2002), and such homogeneous fractions must represent functional SOM pools

that are formed by a specific stabilization mechanism (von Lützow et al., 2007). Various combinations of fractionation methods can be employed for this purpose; one common approach entails physical fractionation by a combination of ultrasonic dispersion and either density or particle size separation prior to chemical fractionation (von Lützow et al., 2007, Sohi et al., 2001, Christensen, 1992).

1.3.1.1 Physical fractionation

The concept of physical fractionation of SOM entails subdividing SOM into different pools according to physical properties; this emphasizes the implication that spatial location is a key factor in determining OM turnover (Balesdent, 1996), since accessibility is prerequisite for decomposition (von Lützow et al., 2006). However, it is impracticable to separate precisely all and only SOM of the same reactivity or specific age, Christensen (1996) and Elliott et al. (1996) suggested that it is rather more convenient to model the measurable than to measure the modelable pools. Sohi et al. (2001) stated that an ideally suitable fractionation procedure should isolate SOM that differs significantly in chemical properties among different pools, thereby suggesting credible differences in reactivity and turnover rates. Physical fractionation techniques involve a combination of either density fractionation or particle size fractionation and ultrasonic soil dispersion (Sohi et al., 2001, Christensen, 1992).

1.3.1.2 Density fractionation

The method of density fractionation of samples serves to isolate the free and occluded particulate OM fractions prior and after dispersion, respectively (Sohi et al., 2001, Golchin et al., 1994). Several high density solutions have been proposed for this purpose, including sodium polytungstate (Na_6 ($H_2W_{12}O_{40}$).H_2O) (Golchin et al., 1994) and sodium iodide (NaI) (Sohi et al., 2001) solutions. The free OM fraction is isolated by simply suspending a sample of soil in a high density solution and decanting the light (floating) material, the remaining soil residue is disaggregated by sonification to liberate the occluded OM (Sohi et al., 2001, Golchin et al., 1994). Both recovered fractions are washed clean and preserved for chemical analysis. Furthermore, according to Sohi et al. (2001), it is perceptible that the recovery of intra-aggregate OM is highly sensitive to separation density and greatly influenced by the dispersion energy, with greatest recovery attained at highest solution density

and dispersion energy (1.80 g cm^{-3} and 1500 $J.g^{-1}$ respectively). Sohi et al. (2001) further suggested that the sensitivity of the intra-aggregate fraction and relative insensitivity of the free OM to separation density could probably be accredited to its tight association with organo-mineral particles and loose association with heavy organo-mineral particles, respectively.

1.3.1.3 Ultrasonic dispersion (sonification)

Adsorption of OM substrates on mineral surfaces and occlusion within soil aggregates, at sites spatially inaccessible to microorganisms, provides sizeable amount of physical protection (Christensen, 1992, Golchin et al., 1994). Mikutta et al. (2005) suggested that some organic constituents for instance lignin-derived, low molecular-weight carboxylic acids, and N-containing compounds might escape chemical destruction if closely associated with minerals. Physical disruption of soil aggregates exposes OM formerly inaccessible to microbial attack (Rovira and Greacen, 1957). Physical disruption of soil aggregates is necessary for the purpose of liberating the occluded OM prior to isolation with heavy solutions, and can be virtually simulated by ultrasonic dispersion.

1.3.1.4 Chemical fractionation

Stable organic matter can be defined as the material that survived destruction by chemical treatment, the residual organic carbon seems to represent largely refractory OM (Mikutta et al., 2006). Hydrogen peroxide (H_2O_2), sodium hypochlorite (NaOCl) and disodium peroxodisulfate ($Na_2S_2O_8$) are the most acknowledged chemical reagents for this purpose (Mikutta et al., 2005). Various methods have been studied and reviewed e.g. Balesdent (1996); Helfrich et al. (2007) and Mikutta et al. (2005) for isolation of stable OM fractions using oxidizing chemicals. Treatment with oxidizing reagents such as H_2O_2, NaOCl, and $Na_2S_2O_8$ was suggested to mimic biodegradation, in the sense that oxidative treatments preferentially remove the less protected SOC whilst subsequently enriching the older SOC component (Esterhues et al., 2003, Mikutta et al., 2006). Treatment with NaOCl and $Na_2S_2O_8$ was considered more effective than with H_2O_2 in isolating SOC (Mikutta et al., 2005). On the contrary, treatments with H_2O_2 and $Na_2S_2O_8$ were rated the most efficient according to Helfrich et al. (2007) on his evaluation of the efficiency of different

chemical fractionation methods by treatment with H_2O_2, NaOCl, (NaOCl + HF), $Na_2S_2O_8$ and stepwise hydrolysis on soils under different land use.

Table 1.1 General properties of the different organic C fractions isolated by physical fractionation (Helfrich et al., 2007, Mikutta et al., 2006).

Organic C fraction	General properties
1. Free particulate organic matter (FPOM)	Light, predominantly labile and typically < 1.6 g cm^{-3}
2. Intra-aggregate organic matter	Occluded within soil aggregates Separated by ultrasonification at 1500 J g^{-1} soil and density fractionation
3. Mineral organic matter (MOM)	Heavy, mineral-bound fraction Organic matter remaining in the heavy fraction after removal of the intra-aggregate OM
3.1. Stable OM	Mineral-protected Digested by oxidation with 10 % H_2O_2 and most other oxidants
3.2. Chemically Stable OM	Recalcitrant fraction, resistant to oxidation with 10 % H_2O_2 and most other oxidants

1.3.2 Characterization of SOM fractions

Solid-state Cross polarization magic angle spinning ^{13}C Nuclear Magnetic Resonance (CPMAS ^{13}C NMR) spectroscopy will be efficiently employed to study and investigate the variation in chemical composition of the isolated fractions. ^{13}C NMR techniques permit direct chemical characterization of organic materials in soil (Baldock et al., 1992). Application of NMR spectroscopy in this study will be useful in identifying ^{13}C carbon atoms of the isolated SOM fractions from the density separation.

1.4 CONCLUSION

Climate is generally considered the most profound soil-forming factor governing the SOM content; consequently most SOM studies on C stabilization have targeted temperate and tropical regions, with little recognition of arid and semi-arid regions. Arid and semi-arid regions constitute a substantial portion *ca*. 30-40 % of the global land surface (Emanuel et al., 1985, Schantz, 1956, and White et al., 2009). Thus quantification of soil carbon stocks contained in these ecosystems provide an indispensable means of evaluating the potential of soils to serve as an ultimate sink for terrestrial organic carbon. Understanding of specific mechanisms at play in the stabilization of carbon in semi-arid soils is of utmost importance, given the potential expansion of their proportion with progressing climate change.

CHAPTER TWO

DISTRIBUTION OF ORGANIC AND INORGANIC CARBON WITH SELECTED SOIL PROPERTIES IN THE SPEKBOOM THICKET

2.1 INTRODUCTION

Organic matter content is widely variable in soils and the extent of its variability is determined by the relative intensity of the influence exerted by certain soil forming factors. Although the soil organic matter contributes only a little to the total soil mass (Baldock and Nelson, 2000), it can exert a strong influence on various soil properties and ecosystem functioning. Management practices, climate, and biota (vegetation and soil organisms) are the most influential soil forming factors in soil organic carbon (SOC) dynamics, although topography and soil mineral composition, as well as the frequency of various catastrophic events e.g. fire, flooding etc. also play an important role.

Generally, a common trend is that SOC content increases along the rainfall gradient and against the temperature gradient, from warm dessert to cooler forest ecosystems (Woodward, 1987, Post et al., 1982). Semi-arid regions are characterized by high temperatures and low annual precipitation, as a norm, and soils from such regions are commonly known to contain low quantities of organic carbon, often less than 10 g C kg^{-1}. In the face of such climatic constraints, the spekboom thicket produces enormous amounts of litter ca. 450 g m^{-2} $year^{-1}$ and an average of 52 g C kg^{-1} soil in the 0-10 cm (Mills et al. 2005). However, spekboom is very susceptible to heavy browsing by domestic livestock and wild animals, slowly transforming the thicket from its naturally dense intact state to a fairly bare savannah/desert-like system (Lechmere-Oertel, 2003). A sizeable portion of the thicket is already in a severely degraded state.

Previously, literature regarding SOC has been disproportionately directed towards temperate and tropical regions of higher latitudes, typically characterized by higher annual precipitation and cooler summer temperatures; whilst the gaps in knowledge pertaining to SOC dynamics in arid and semi-arid regions remain uncharted. Several studies have been carried out on the interactions between vegetation type and SOC

dynamics in arid and semi-arid ecosystems in North America, for instance, Martínez et al. (2007), White et al. (2009), and Rasmussen and White (2010). Similar to McCulley et al. (2004), they found significant variations in SOC with vegetation type, with greater SOC content under woody vegetation compared to the grassy sites.

Previous research on spekboom thickets e.g. Lechmere-Oertel (2003), Lechmere-Oertel et al. (2008), Mills et al. (2005), and Mills and Fey (2004) have discovered the extraordinary capacity of spekboom to store large quantities of carbon in the soil through its high rates of litter production and various other attributes. However, it remains to be determined, using state of the art measuring techniques, how much of the carbon contained in these soils is organic as opposed to that which is inorganic. It is also unclear whether the remarkable C accumulation in these soils is explicitly due to the spekboom plant (*Portulacaria afra*), and whether there are any differences in the soil chemistry under spekboom and nonspekboom vegetation. The extent of hydrophobicity in these soils has also never been examined, which has a strong impact on soil water infiltration but also retention.

Therefore, the main objective of this study was to quantify the organic C and inorganic C stocks in the top 50 cm of the intact (directly under spekboom and also nonspekboom vegetation) and degraded thicket soils. A further aim was to examine the impact of browsing-induced transformation and vegetation type on selected soil properties (pH, EC, water-soluble and exchangeable cations, and hydrophobicity) to gain a better understanding of the chemical processes in these soils.

2.2 MATERIALS AND METHODS

2.2.1 Experimental design and field sampling

The study was conducted in a thicket ecosystem, characterized by a matrix of various vegetation types, including *Euclea undulata, Pappea capensis, Portulacaria afra,* and *Rhus longispina* species, though largely dominated by *P. afra*, a succulent shrub native to Africa, locally known as spekboom. The thicket under study is located at Rhinosterhoek farm (33.4°S, 25.0°E), found between Uitenhage and Kirkwood in the Eastern Cape, South Africa. This is within the semi-arid climatic zone; *ca*. 250 – 500 mm rainfall evenly distributed throughout the year (Fig. 2.1). The farm has intact, mature spekboom thicket which is fenced off from grazing animals, as well as open areas where the spekboom has been overgrazed and replaced by annual grasses. Based on visual observations in the field; the spekboom component of the original intact thicket has been completely removed from the open area landscapes, leaving behind sparsely scattered short-lived grasses and remnant perennial trees, as depicted in Fig. 2.2. These open areas largely resemble savanna ecosystems (Lechmere-Oertel et al., 2005).

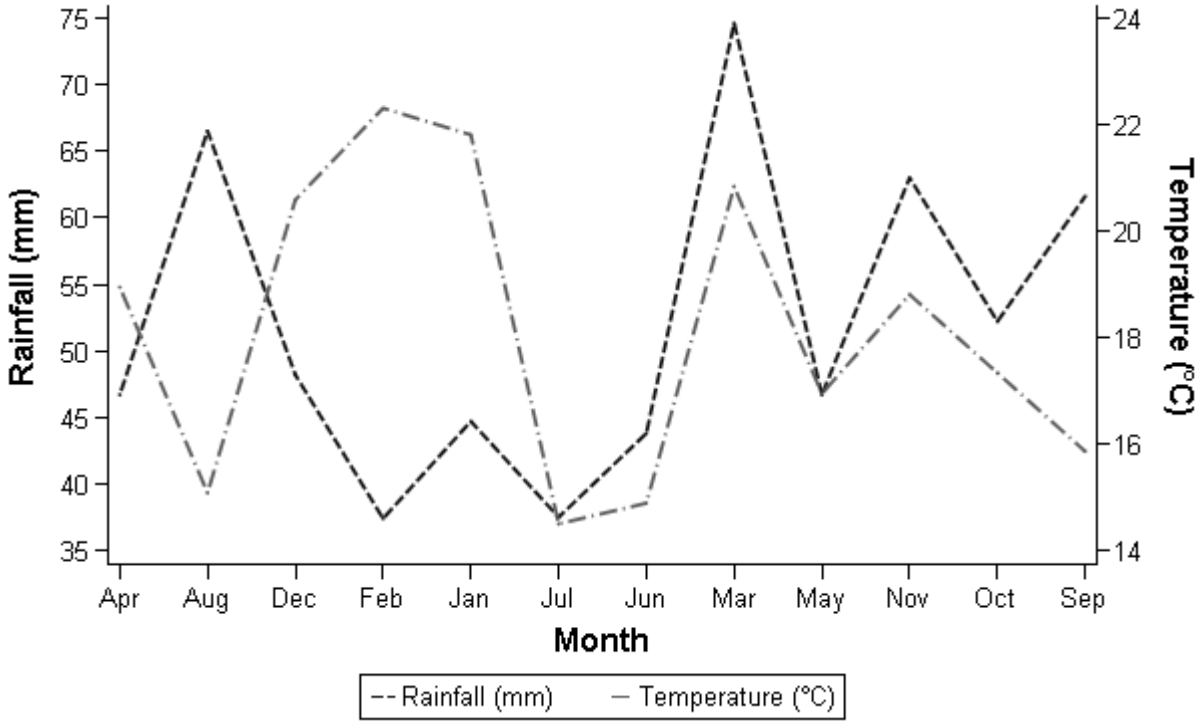

Fig. 2.1 Climatic data from a nearby weather station in Kirkwood town, located *ca*. 30 km from the experimental sites (South African Weather Bureau).

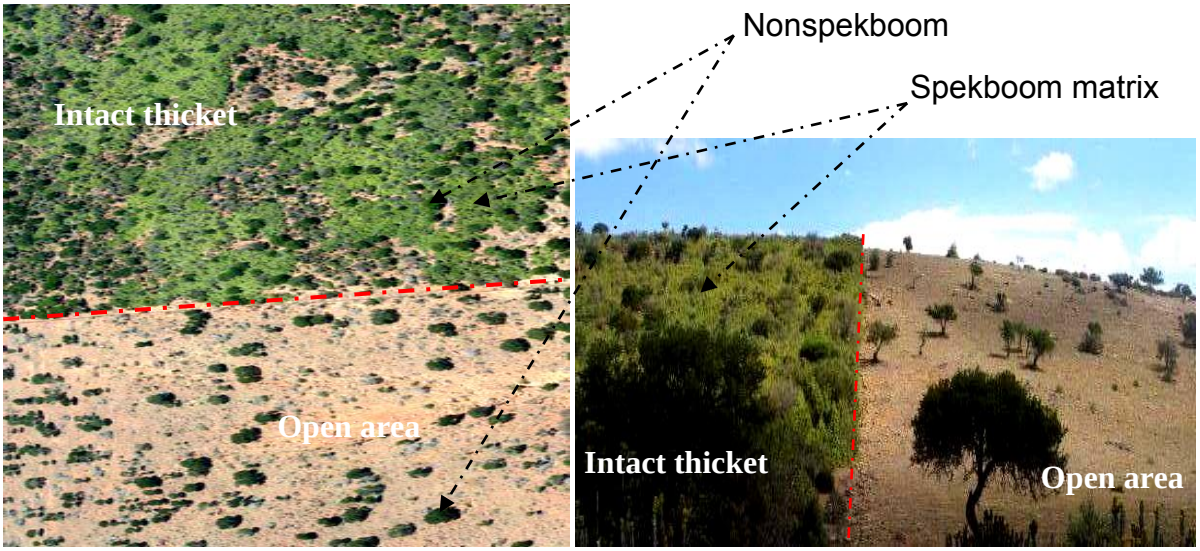

Fig. 2.2 Fence-line transects (red dotted line) depicting the intact vs. transformed open area sites (*pictures by Mills et al. 2005*).

Soil parent material in the region is a mixture of weathered bedrock and transported material, mostly likely due to the colluvial or alluvial (possibly periodic flash floods which is not uncommon in the karoo) manner in which these soils have formed. The mixture comprises of colluvial deposits, including Bokkeveld shales, Uitenhage conglomerates and mudstones (Mills, 2003).

Twelve fence-line transects were randomly selected across the thicket. Three profile pits were then excavated at each of the twelve sites; one directly under spekboom vegetation and one under another type of vegetation “non-spekboom” but within the intact thicket, and another in the open area. The sampling technique resembled a typical natural snapshot experiment design (Diamond, 1986), where sites were allocated according to vegetation type (spekboom, nonspekboom, and open “grassy” sites), and the fence-line transects as replications of each site. The three sites were located in close proximity (within 2 meters) from each other at each transect.

Organic (litter) layers were collected prior to soil sampling in 50 × 50 cm^2 quadrats directly under spekboom and in nonspekboom canopies, the area was doubled to 100 ×100 cm^2 in the open area since only little amounts of litter was present. Thereafter, mineral soil layers were collected at 10 × 10 cm^2 quadrats for four successive depth intervals 0-5, 5-15, 15-25 and 25-50 cm. A separate set of samples was collected in 20 cm increments for the determination of bulk density. Bulk density was determined using the sand method (Blake and Hartge, 1986), whereby, the

volume of excavated soil was determined by measuring the mass of coarse sand (with a known bulk density) required to fill the volume of the sampled area. A calcrete layer was often encountered below 40 cm. Soil morphological characteristics were recorded in the field, and the soil was classified according to the taxonomic system for South Africa (SSSA, 2001). Most of the soils were classified to the Addo, Augrabies, and Brandvlei soil forms, corresponding to neocalcic and calcaric Calcisols of the Cumulic Subgroup (Fey, 2010) in the World reference Base classification system (images depicted in Fig. 2.3). Another distinct visual observation noted during sampling was the thick litter layer and an extensive root network, which seemed to be characteristic of thicket sites but absent in the open areas as illustrated in Fig. 2.4 below.

All laboratory soil analyses were performed on air-dried, sieved (< 2 mm) soil unless otherwise specified. Foliar samples of the different vegetation species occurring in the thicket were randomly sampled as a representative of the source vegetation, however, foliar material could not be sampled for open area sites, as only dead grasses and scattered remnant trees were found on these sites at the time of sampling. The samples comprised of a mixture of young, mature, shaded, and exposed leaves of each species. Samples were then bulked into composite samples of spekboom and nonspekboom species for subsequent analyses.

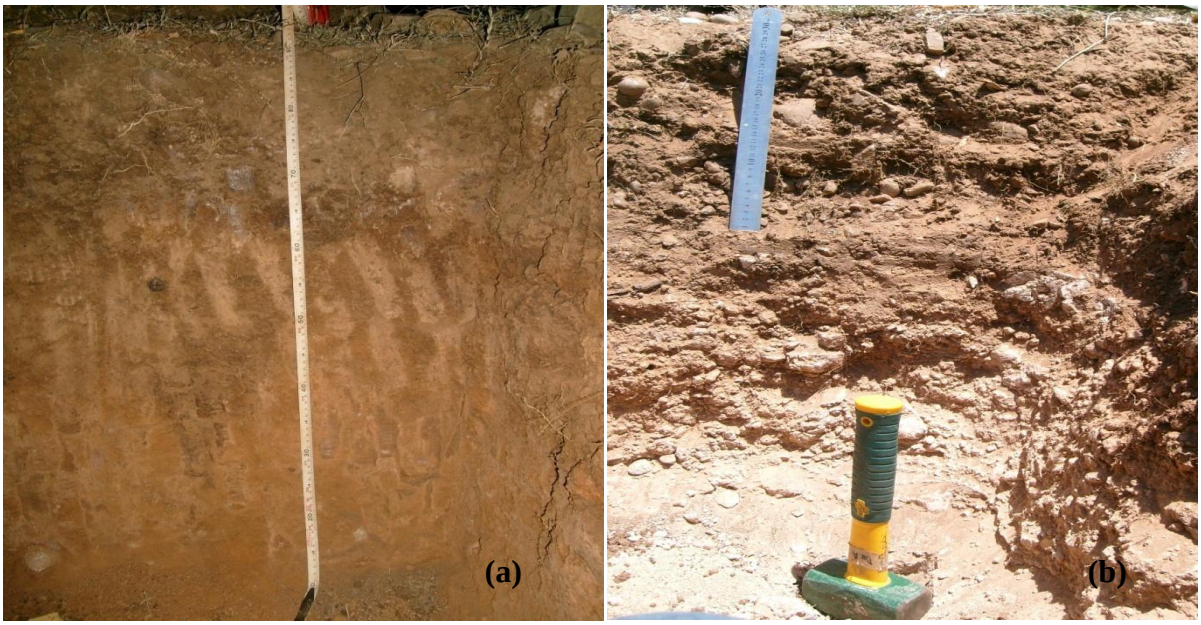

Fig. 2.3 Soil profile forms that dominated most of the thicket area surveyed, (a) deep, well-drained **Augrabies** and (b) relatively shallow, calcareous **Addo**.

Fig. 2.4 Contrasting differences in amounts of litter and root network directly underneath spekboom and open area at the same fence-line transect.

2.2.2 Laboratory analyses

Total C and N were determined using dry combustion (Euro Vector elemental analyzer). Several attempts were made to identify an accurate quantitative method to distinguish between the organic and inorganic carbon fractions. The first attempt during preliminary study aimed to remove the carbonates with dilute phosphoric acid (H_3PO_4) (Nelson and Sommers, 1996) prior to C analysis; the estimated residual C after H_3PO_4 treatment is considered to be organic. The H_3PO_4 pre-treatment appeared to hydrolyze some of the organic C, as shown by a sizeable loss of N (25-33 %).

A gentler acid fumigation method (Harris et al., 2001) was then used, which did not result in a significant N loss in the samples. The method entails wetting 30 mg of finely ground (ball-milled to less than 1 mm) soil to field capacity with a 50 µL droplet of distilled water, then placing the samples in a tightly sealed dessicator chamber containing a beaker of concentrated 32 % HCl. The HCl vapour then dissolves in the wet sample while slowly digesting the inorganic carbonates. To determine the optimal fumigation time, two soil samples (spekboom vs. non-spekboom) with high pH and total carbon content were treated for successive number of hours; 12, 24, 72, 48, 96, 120 and 144 hours. It was established that 5 days (120 hours) is sufficient time for complete carbonate dissolution without loss of the N fraction.

Soil pH was measured in distilled water and in 1M KCl at a 1:2.5 soil to solution ratio (White, 1997) after shaking the suspensions on a horizontal shaker for 30 min. and allowing 30 min. standing time before the reading was recorded. The concentration of water soluble basic cations and EC was determined in a 1:5 extract (Rhoades, 1982), prepared in a similar manner as the pH samples. Soil particle size fractions were determined according to the wet sieving and pipette method, and soil texture was deduced from a textural triangle chart (Gee and Bauder, 1986). The bulk density soil samples were oven dried to a constant mass, weighed, and separated from coarse (> 2mm) fragments by wet sieving. The volume of the residual rock fragments was determined using the cylinder (water displacement) method (Blake and Hartge, 1986) and then deducted from total volume of soil for bulk density calculations. Exchangeable basic cations (Ca, Mg, Na, and K) were determined according to the modified method for calcareous soils by Normandin et al. (1998) using 1M NH_4OAc at pH 8.5 in 1:5 soil to solution ratio. This method is suitable for determination of exchangeable cations in calcareous soils as it avoids dissolution of carbonates often encountered with the original 1M NH_4OAc at pH 7 method. A total of 1:10 (m/v) ratio (5 g soil and 50 mL solution) was used in combination with horizontal shaking at 3000 rpm for 30 minutes and centrifuging at 2000 rpm for 10 minutes, respectively. Thereafter, the supernatant was filtered through Whatman 40 filter paper and analyzed.

Soils were also tested for hydrophobicity with the water-drop penetration time (WDPT) method (Bisdom et al., 1993), which entails categorizing soils into hydrophobicity classes according to how long it takes a 50 µL droplet of water to break the surface tension and completely infiltrate through the soil surface. Samples were equilibrated at ambient temperature and relative humidity overnight in the laboratory prior to the analysis. Samples were then assigned to one of the five WDPT classes depending on the duration of resistance to wetting. WDPT < 10s is considered hydrophilic and ≥ 10s hydrophobic, 10s-60s represents the slight hydrophobicity class, whereas 60-600s and 3600s represent the strong and severe hydrophobicity classes respectively. WDPT longer than an hour (3600s) was classified as extremely hydrophobic. One sample was duplicated for every 36 samples during each of these analyses, to ensure optimal accuracy.

Composite foliage and litter samples were thoroughly rinsed three times with distilled water and oven dried at 40 °C for 48 hours prior to analysis for C & N content by dry combustion with the Elemental analyzer. Basic cation (Ca, Mg, Na, & K) composition was also determined for the composite foliage samples.

2.2.3 Statistical analysis

Means and standard errors of the different variables were computed per site for each depth, and then plotted as bar charts on Microsoft excel. Further statistical analyses were performed and interpreted according to the one-way ANOVA procedure using SAS Enterprise Guide (version 4 and 9.1) software. Differences between means were determined by the least significant difference according to Bonferroni t-test.

2.3 RESULTS AND DISCUSSION

2.3.1 Organic and inorganic C content

The most significant differences in total, organic or inorganic C content between sites were located within the top 25 cm from soil surface (Fig. 2.5). Organic C content was closely similar and did not differ significantly in the top 25 cm; 62.4 ± 4.9 vs. 58.5 ± 6.2 g C kg^{-1} at 0-5 cm, 42.1 ± 3.8 vs. 44.5 ± 4.3 g C kg^{-1} at 5-15 cm, and 33.7 ± 3.3 vs. 32.9 ± 3.5 g C kg^{-1} at 15-25 cm for spekboom and nonspekboom sites, respectively (Table A2.1, appendix). This phenomenon could possibly be attributed to the dense interwoven nature of the thicket with clustered canopies such that spekboom and nonspekboom species are co-located as a matrix of inter-tangled canopies within the intact thicket, as depicted in Fig. 2.2. As had been previously shown (Mills and Fey 2004), the degraded, open area soils contained significantly lower total and organic C content especially in the top 25 cm of the profile; *ca*. 34.0 ± 2.9, 26.4 ±1.9, and 21.8 ± 2.5 g C kg^{-1} at 0-5, 5-15, and 15-25 cm respectively (Table A2.1, appendix).

Mean SOC content was nearly twice as high in the topsoils (0-5 cm) of intact spekboom (62.4 g C kg^{-1}), and nonspekboom (58.5 g C kg^{-1}) sites relative to open grassy sites (34.0 g C kg^{-1}) (Table A2.1, appendix). These SOC values are comparable to those previously reported by Mills and Fey (2004) in preceding soil C studies on thicket transformation effects on soil C, their findings indicated *ca.* 30 vs. 56 g C kg^{-1} soil at 0-10 cm for open and spekboom sites, respectively. Our results

are also quite comparable to other studies on vegetation controls on SOC dynamics, for instance White et al. (2009) indicated almost twice as large SOC amounts under woody vegetation types (46.7 and 30.4 g C m^{-2} for mesquite and creosote vegetation, respectively) relative to grassland sites (24.4 g C m^{-2}) in an arid hyperthermic ecosystem. Similarly, McCulley et al. (2004) also found more than double the amounts of organic C under encroaching woody pant communities (*ca*. 3.4 kg C m^{-2}) relative to adjacent grassland sites (*ca*.1.7 kg C m^{-2}) in a subtropical savanna ecosystem.

Substantial amounts, although in varying quantities, of inorganic C were detected in most soils, but organic C comprised the dominant fraction of total C (78-96 %) regardless of the transformation state or vegetation type (Fig. 2.6). It also appeared that the inorganic C content increased with depth under open sites, whereas in the intact thicket sites it gradually decreased with depth; nonetheless, inorganic C content did not differ significantly between sites (Fig. 2.5 c). On average, mean inorganic C content ranged from 2–9 g C kg^{-1}, comprising approximately 4-22 % of total C (Fig. 2.6).

Soil organic C losses associated with transformation appeared to be more pronounced at shallower depths and gradually diminish below 25 cm. It is evident that transformation of the thicket into an open, savanna-like ecosystem has remarkably reduced the organic C content by approximately 46 % in the top 0-5 cm i.e. 34.0 g C kg^{-1} in contrast to 62.4 g C kg^{-1} from spekboom sites. Similarly, 5-15 and 15-25 cm indicated *ca*. 37 and 35 % decline in SOC content, respectively. The changes in plant species composition, specifically, spekboom disappearance and subsequent substitution by annual grasses signifies a huge contrast in standing biomass, and hence nutrient concentrations, and organic C quality as well as quantity between the intact thicket and open areas. Continuous grazing and consumption of aboveground biomass by domestic and wild animals in the open areas drastically reduce the amount of litter that falls on the soil surface, and inevitably culminates in lower soil carbon and nutrients.

Although SOC content was not species-specific within the thicket, from these results and previous research on spekboom thickets, it is evident that spekboom represents a critical component to SOC storage in the thicket, most probably attributable to its

capacity to produce enormous amounts *ca.* 2500 kg ha^{-1} yr^{-1} of litter (Lechmere-Oertel, 2003). Furthermore, its dense canopy structure effectively shields the soil surface from erosion factors and abundance justifies its esteem. Lechmere-Oertel (2008) estimated that the thicket in its intact state can produce up to *ca*. 4100 kg ha^{-1} yr^{-1} on a landscape scale, relative to 2880 kg ha^{-1} yr^{-1} litter after transformation.

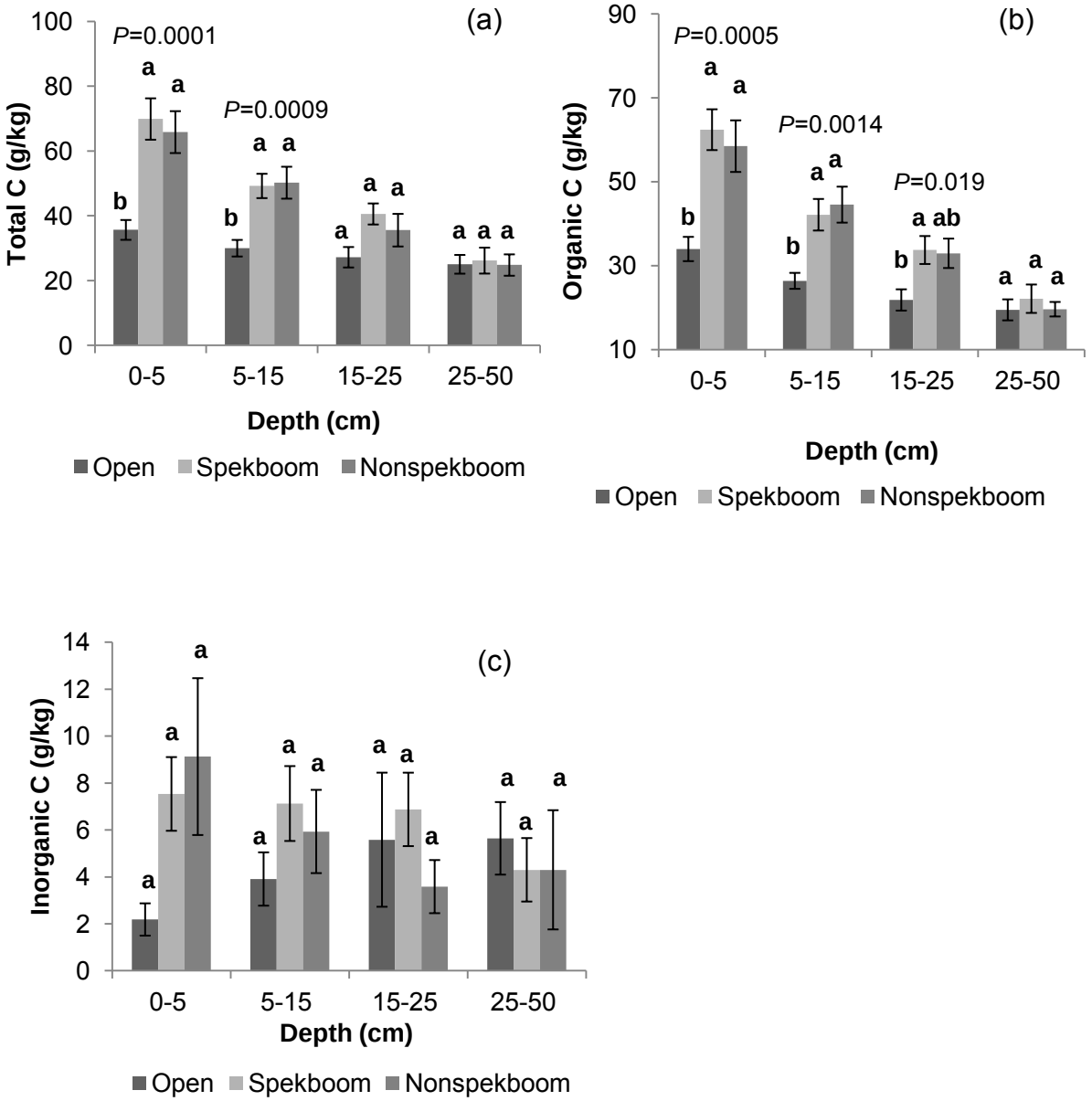

Fig. 2.5 Vertical distribution of (a) total, (b) organic, and (c) inorganic carbon between sites.

Note: Error bars represent standard error, and alphabetic letters denote statistical differences between sites at a specific depth, according to the Bonferroni's least significant difference (lsd) of means at α = 0.05. Similar letters indicate lack of significant differences.

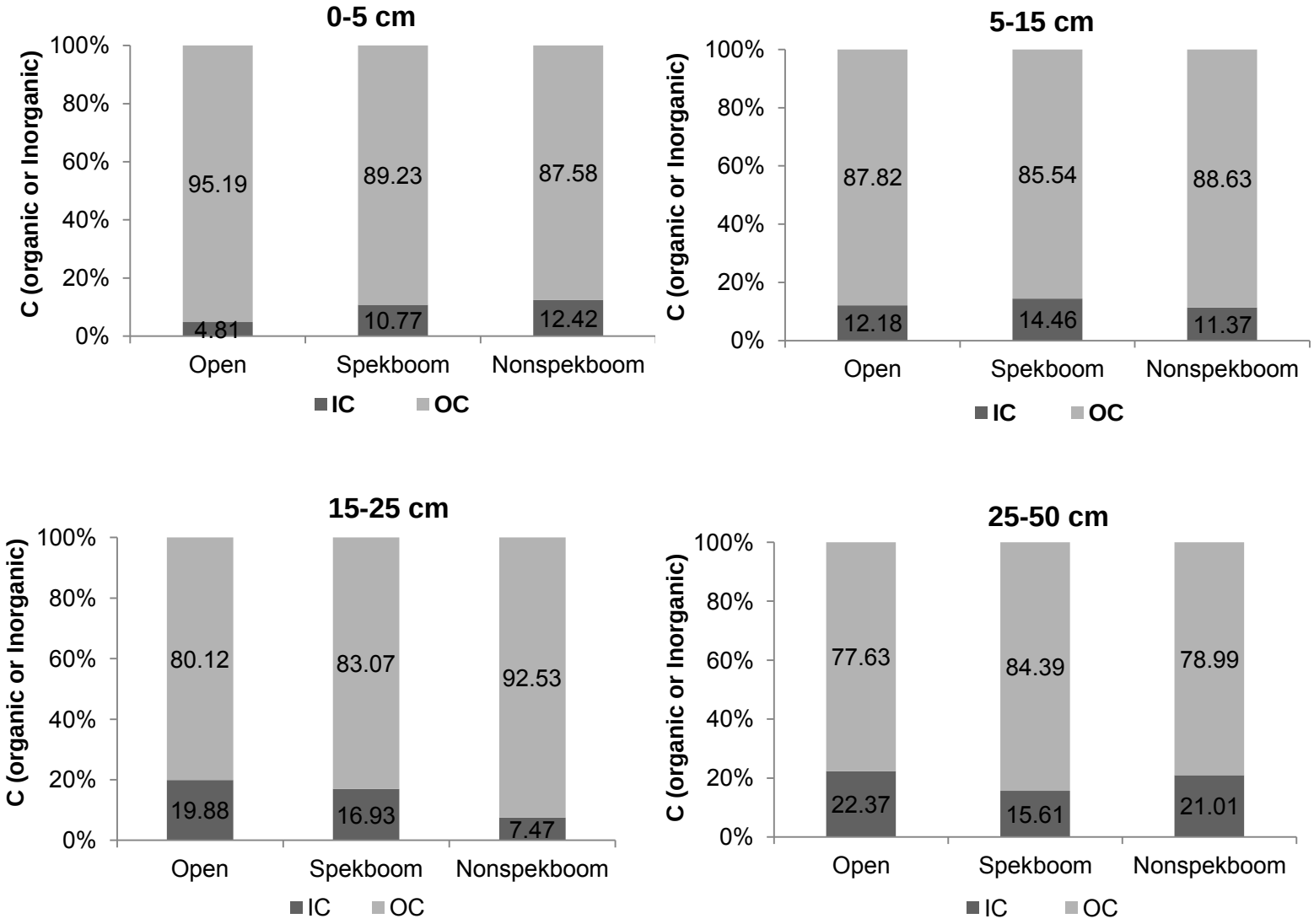

Fig. 2.6 Distribution of organic and inorganic carbon between sites and relative contribution to total carbon .

In natural ecosystems such as this, the amount of organic C contained in soil tends towards equilibrium as a function of the balance between the rates of organic C additions and losses, as dictated by the soil forming factors. These results indicate that the clearing of indigenous vegetation cover due to intense grazing by livestock and wild herbivores, has altered this natural balance of input and output ecosystem processes through biomass recycling.

The observed patterns in organic C distribution across the fence-line contrasts can be ascribed to several causal processes induced by the transformation, as hypothesized by Mills and Fey (2004), Mills et al. (2005), and Lechmere-Oertel et al. (2005, 2008), who have carried out extensive research on succulent spekboom thickets locally. The first and probably most prominent of these processes is vertical C cycling and incorporation into soil in the form of plant residues, evidenced by the thick intact litter layer in the vegetated sites as depicted on Fig. 2.4 a, as opposed to reduced biomass production in open areas. Mills et al. (2005) reported up to 75 % losses of aboveground biomass in transformed sites. Lechmere-Oertel (2003) also found there to be a significant interaction between transformation status i.e. intact vs. degraded landscapes and certain vegetation species; his findings established that *Euclea undulata* and *Papea capensis* produced approximately 60 and 55 % less litter, respectively, following transformation. In addition to aboveground litter inputs, proliferation and death of perennial plant roots is also expected to contribute a considerable fraction towards SOC content in the intact thicket.

Vegetation canopy cover and the derivative litter layer regulates soil temperatures through canopy shading and the mulching effect, respectively, this is substantiated by Lechmere-Oertel (2003), he reported 12 °C lower soil temperatures; *ca*. 23.2 °C mean daily maximum temperature measured directly beneath spekboom canopies in the intact thicket, contrasting to 35.1 °C in the open areas. Moreover, the water holding attributes of the litter layer provide the intact thicket with further advantage of prolonged moisture availability after rainfall events, the resultant cooler conditions may appreciably repress abiotic mineralization of organic matter in the intact thicket. Although moist condition may accelerate OM mineralization, the hydrophobic nature of these soils (discussed in detail under subsequent sections) may counteract this effect by exclusion of water.

Based on field observations; bioturbation through the trampling action by grazing animals (kudus, springboks, and goats etc.) can be expected to exert a significant influence on the reflected total C contents in the open area sites, through inevitable dislocation and blowing away of surface litter (and possibly soil material) during the dry season, which is characteristic of semi-arid regions such as this. Soil organic C mineralization is generally parallel with temperature (Post et al., 1982); ultra-violet (UV) photo-oxidation has been reported to accelerate OM mineralization (Schaefer et al., 1985, Moorhead and Callaghan, 1994). Ladd et al. (1985) observed a doubling effect of the rate of C mineralization for an 8-10 °C increase in mean annual temperature. Reduced canopy coverage in the open areas increases direct exposure of litter to high mid-day temperatures and strong UV radiation, typical of arid environments, which may substantially induce abiotic OM mineralization.

In contrast, the structural arrangement of spekboom canopies substantially shade the SOM against abiotic mineralization. Furthermore, the canopy matrix of the intact thicket serves as a wind breaker intercepts rainfall, thereby securely protecting surface OM against erosion factors such as wind, raindrop splash and run off; whereas, increased vulnerability to leaching and/or erosion (overland runoff) may be expected in the open area sites after heavy rainfall events. Significant losses of surface litter and soil may result in this manner; the outcome is already revealing itself, evidenced by a remarkable decline in organic C (OC) on the transformed open area sites.

Thicket transformation (open) and vegetation type (nonspekboom) did not have a significant effect on the OC:N ratio of litter relative to spekboom (Fig. 2.7). However a substantial decline was noted in the top 0-5 cm of open sites, which possibly suggests accelerated mineralization rates, most likely a result of poor residue quality and/or OM loss through erosion. Comparable organic C study of plant material (foliage) and litter across sites demonstrated a gradual decline in organic C content in a vertical direction from foliage > litter; however with significantly higher OC:N ratio under spekboom litter and nonspekboom foliage relative to other sites (Table 2.1). Although litter OC:N ratios reflected only little variation between sites, it can be expected that secondary decomposition products from litter may enhance rather than

reduce litter quality, a phenomenon known as secondary recalcitrance (Sollins et al., 1996; von Lützow et al., 2006) which may reduce rates of decomposition.

The foliage organic C content were closely comparable to those reported on other SOC studies in semi-arid ecosystems e.g. Rasmussen and White (2010), carried out on akin semi-arid climate in Arizona, North America. However, litter and bulk soil organic C values were respectively, nearly twice and ten times higher compared to those reported by Rasmussen and White (2010), this largely suggests a very close relationship between the specific vegetational input in terms of residue quality and most likely quantity as well.

Table 2.1 Vertical distribution of organic C and OC:N ratios according to vegetational inputs (foliage - litter - bulk soil (0-50 cm).

	Site	OC (g/kg)	OC:N
Foliage	Open	n.d	n.d
	Spekboom	404.2 ***b***	19.4 ***b***
	Nonspekboom	443.3 ***a***	20.6 ***a***
Litter	Open	342.6 ***a***	22. 6 ***a***
	Spekboom	343.7 ***a***	27.2 ***a***
	Nonspekboom	336.5 ***a***	19.5 ***b***

n.d = not determined.

Italicized letters denote statistical differences between sites at specific depth according to the least significant difference (lsd) of means at α = 0.05. Similar letters indicate lack of significant differences.

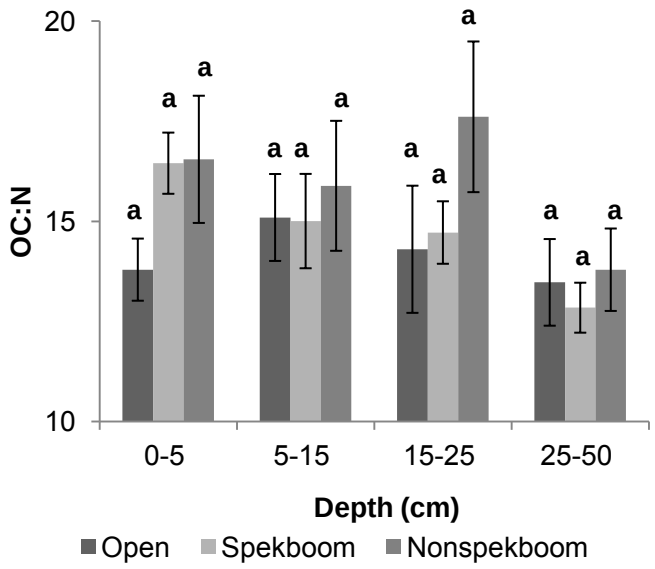

Fig. 2.7 Distribution of OC:N ratio with depth in the upper 50 cm of soil.

Note: Error bars represent standard error, and alphabetic letters denote statistical differences between sites at specific depth according to the least significant difference (lsd) of means at α = 0.05. Similar letters indicate lack of significant differences.

2.3.2 General soil properties

The greatest differences in soil properties between the sites were located within the top 15 cm of the soil profiles. The soils were predominantly alkaline with pH values averaging above pH 7 at all depths in both open and spekboom sites (Fig. 2.8a). On the contrary, nonspekboom soils displayed significantly lower pH values relative to spekboom sites, regardless of the clustered-canopy matrix character of the thicket. High pH conditions encountered under spekboom canopies were strongly correlated to the concentration of water-soluble Na at 0-5 cm (r^2 = 0.69) (Fig. 2.9). Although this correlation was not strong for the open area and nonspekboom soils, such high pH values in open sites most probably reflects the effects of spekboom vegetation that once inhabited these sites during the pre-transformation state, i.e. before land use change towards pastoralism and game farming.

Differences in electrical conductivity were not statistically significant between sites (Fig. 2.8 b). Although transformation did not cause any significant changes in soil pH, the nutrient dynamics were slightly altered, indicated by reduced amounts of total soluble salts, as indicated by electrical conductivity (EC), accompanied by a marked

declines in exchangeable and water soluble basic cations (Ca, Mg, Na, and K) under open area sites (Fig. 2.10 and 2.11 respectively). Although not statistically significant at certain instances, exchangeable Ca, Mg, Na, and K concentrations demonstrated a consistent decreasing trend in the open area relative to spekboom soils, largely expressed in the top 15 cm (Fig. 2.10). Water-soluble Ca and Mg displayed a similar decreasing trend to a depth of 25 cm, whilst Na and K also declined significantly up to 15 cm and 50 cm depth, respectively (Fig. 2.11). However, a sudden increase in EC and soluble cations was noted at lower depths of open area sites, suggesting the downward movement of salts and nutrients, and subsequent accumulation at lower depths through leaching. Within the intact thicket, spekboom soils demonstrated significantly higher soluble salts (EC), exchangeable, and water-soluble basic cations (specifically, Mg, Na, and K) compared to nonspekboom soils (Fig. 2.10 and 2.11).

In this study, vertical recycling of nutrients through litter decomposition can be considered as the primary source of variation in basic cations since the soil parent material was similar between sites. The observed differences in soil nutrient composition between sites were further substantiated by the results from foliage and litter analysis, which facilitated a detailed comparison of intrinsic nutrient composition of the source vegetation from each individual site. Litter composition revealed spekboom to be particularly enriched with basic cations (specifically Mg, Na, and K) in contrast to the adjacent nonspekboom sites, whereas the open area contained the least nutrients (Table 2.2), thus creating a gradual decline in cation concentration in the order of spekboom > nonspekboom > open area. Levels of Ca however, illustrated the opposite trend among the sites; nonspekboom litter appeared to be particularly Ca-rich than other sites, even though no recognizable differences were found relative to spekboom in bulk soils.

In a similar fashion, spekboom foliage was found to contain significantly more Mg, Na, and K relative to nonspekboom species (consistent with bulk soil and litter trends), whereas, Ca content was evidently higher under nonspekboom species, as predicted by preceding litter nutrient trends (Table 2.2). Furthermore, it has been found that spekboom has higher litter production rates compared to other species (nonspekboom) occurring in the thicket (Lechmere-Oertel, 2003, Mills et al., 2005).

This possibly explains the significantly higher concentrations of basic cations under spekboom soils relative to other sites.

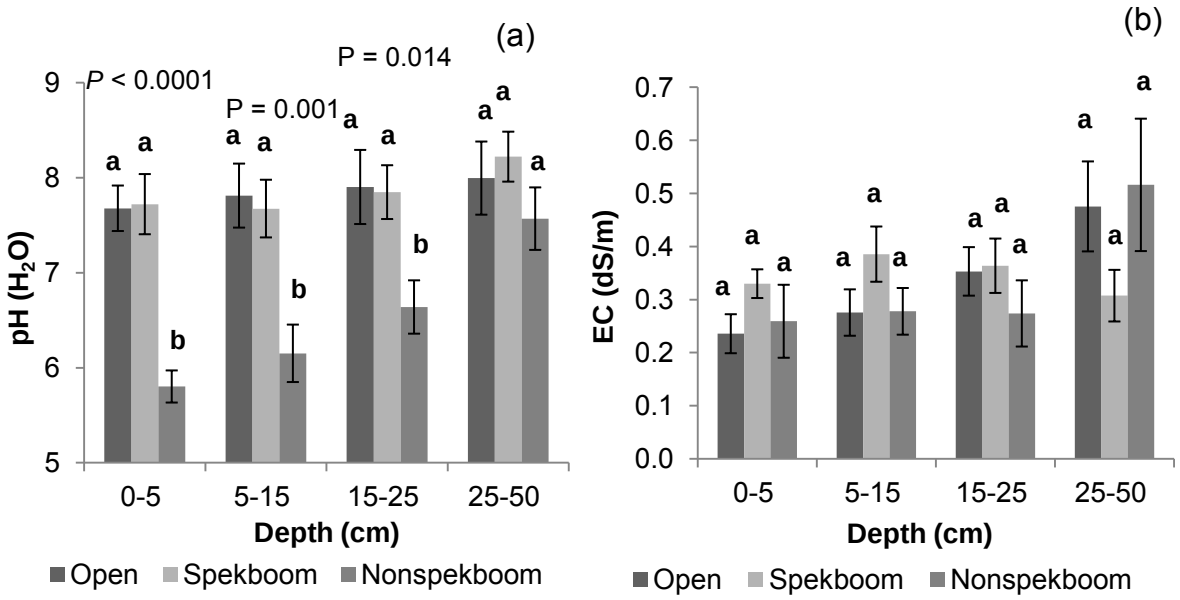

Fig. 2.8 Influence of transformation and vegetation type on (a) soil $pH_{1:2.5}$ and (b) $EC_{1:5}$.

Note: Error bars represent standard error, and alphabetic letters denote statistical differences between sites at specific depth according to the least significant difference (lsd) of means at α = 0.05. Similar letters indicate lack of significant differences.

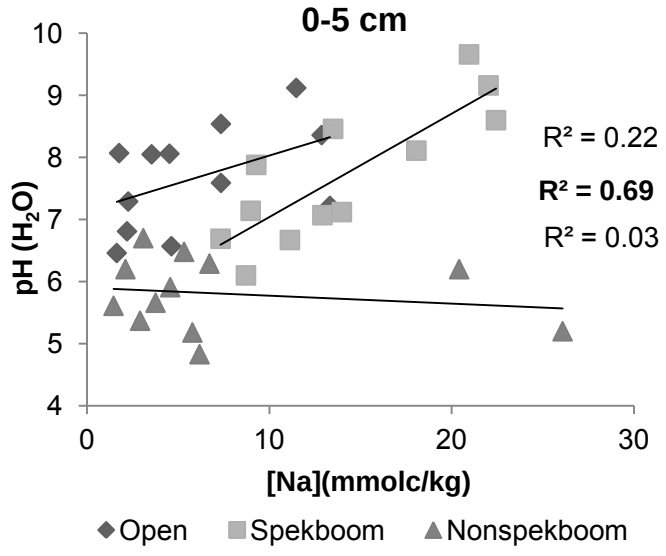

Fig. 2.9 Relationship between water-soluble Na and pH (H_2O) at 0-5 cm.

Note: r^2-values are arranged in a vertical order of open, spekboom, and nonspekboom.

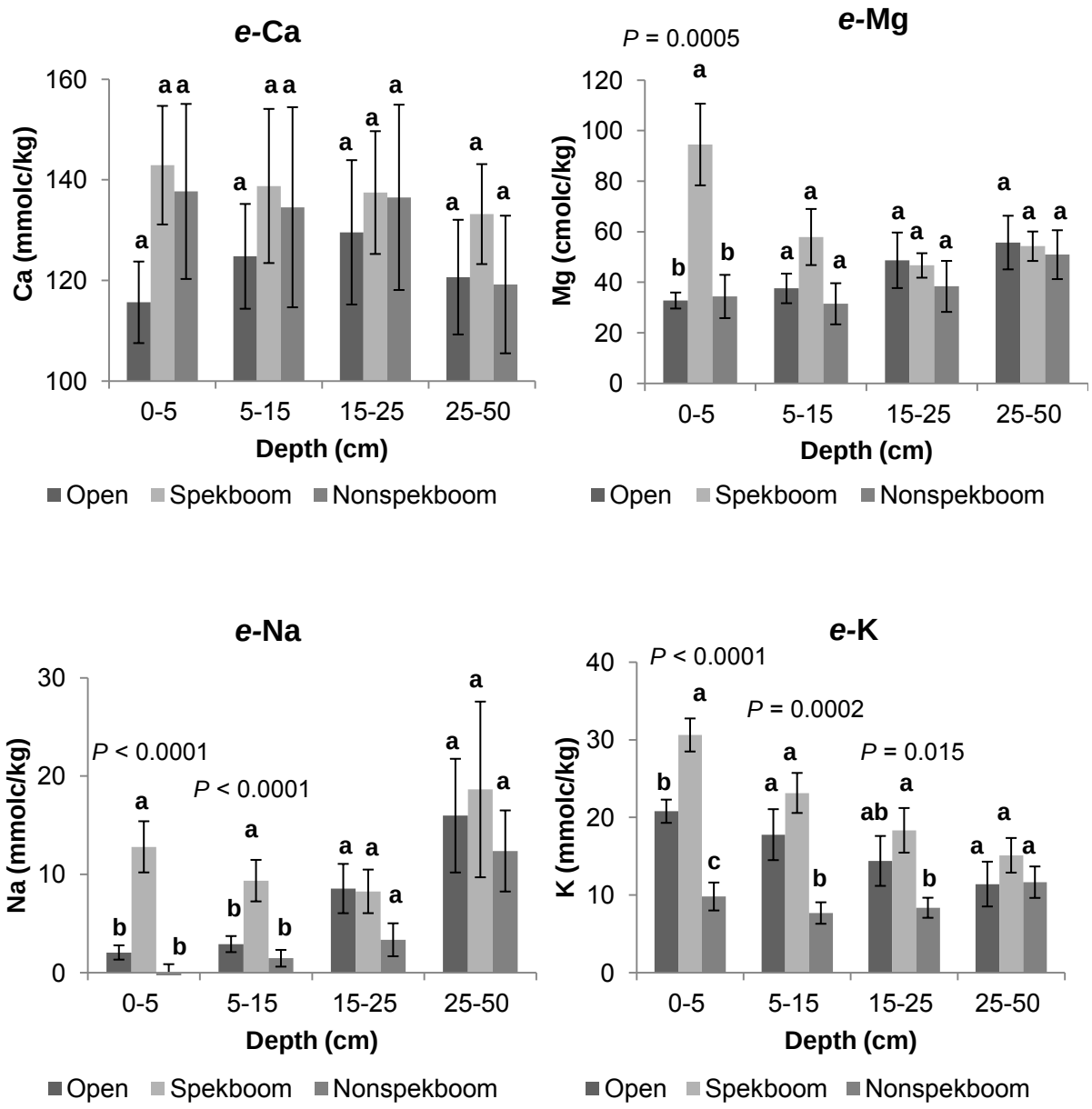

Fig. 2.10 Vertical distribution of exchangeable bases (Ca, Mg, Na, and K) among the different sites. *e* = exchangeable.

Note: Error bars represent standard error, and alphabetic letters denote statistical differences between sites at specific depth according to the least significant difference (lsd) of means at α = 0.05. Similar letters indicate lack of significant differences.

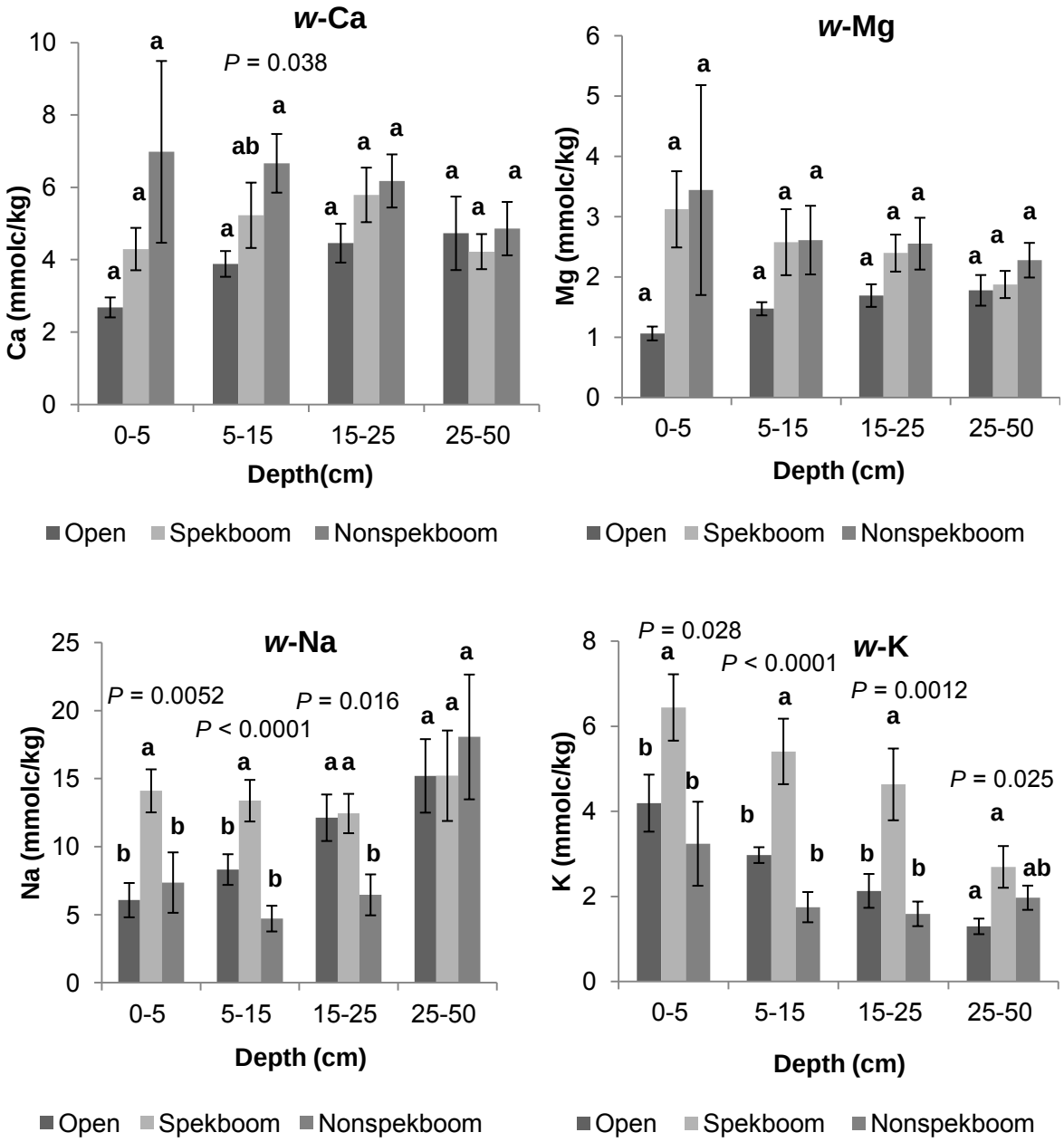

Fig. 2.11 Water-soluble nutrients (Ca, Mg, Na, and K) with depth between sites (open, spekboom, and nonspekboom). *w* = water-soluble.

Note: Error bars represent standard error, and alphabetic letters denote statistical differences between sites at specific depth according to the least significant difference (lsd) of means at α = 0.05. Similar letters indicate lack of significant differences.

Table 2.2 Foliar and litter nutrient composition of the sampled sites.

		Depth (cm)	Open	Spekboom	Nonspekboom
Foliage	%Ca	0-5	n.d	0.5 ***b***	1.7 ***a***
	%Mg	5-15	n.d	0.7 ***a***	0.5 ***b***
	%Na	15-25	n.d	1.4 ***a***	0.1 ***b***
	%K	25-50	n.d	1.8 ***a***	0.9 ***b***
Litter	%Ca	0-5	1.7 ***b***	3.2 ***a***	3.5 ***a***
	%Mg	5-15	0.2 ***b***	1.5 ***a***	0.3 ***b***
	%Na	15-25	0.05 ***b***	0.4 ***a***	0.07 ***b***
	%K	25-50	0.2 ***b***	0.6 ***a***	0.2 ***b***

n.d = not determined. Alphabetic letters denote statistical differences between sites at specific depth according to the least significant difference (lsd) of means at α = 0.05. Similar letters indicate lack of significant differences.

2.3.3 Soil hydrophobicity

Soils displayed variable wettability, from hydrophilic (wettable) to hydrophobic (water repellent). A large number of studies e.g. DeBano et al. (1970), Hallett et al. (2001), and Giovannini et al. (1983, 1987) have investigated the effects and environmental impacts of soil hydrophobicity but mostly on acidic soils and fire affected areas as they are generally considered vulnerable to this phenomenon.

In this study we found intact thicket soils (spekboom and nonspekboom) to be the most strongly water repellent, especially in the top 15 cm, on the contrary; open area soils were seemingly hydrophilic (non-hydrophobic) in the top 0-5 cm (Fig. 2.12), as determined by the persistence of a water droplet on the soil surface according to Table 2.3 below. The open area topsoils (0-5 cm) were less prone to hydrophobicity, which persisted on average only for *ca.* 2.4 seconds compared to 16.04 and 11.66 minutes for spekboom and nonspekboom sites, respectively (Fig. 2.12). Furthermore, spekboom soils appeared to be significantly more hydrophobic relative to other sites even at 15-25 cm (Fig. 2.12). Although a considerable increase in hydrophobicity was noted below 5 cm under open areas, hydrophilic soils comprised the largest part of open areas, ranging from 67 % for subsoil (25-50 cm) up to *ca*. 92 % in the top 0-5 cm (Fig. 2.13).

Thicket transformation appears to have greatly affected the water repelling characteristics of open area sites. This is evidenced by low WDPT values and rarity of hydrophobicity especially on topsoils. Hydrophobicity was found to be less frequent and of lower persistence in the open area sites, only 8 % (i.e. only 4 of 48 samples) were classified as slightly hydrophobic (10-60s), and none of these soils were assigned to higher hydrophobicity classes (Table A2.4, appendix). On the contrary, 75 and 67 % frequencies were obtained for spekboom and nonspekboom topsoils, respectively. Spekboom and nonspekboom were largely similar and ranked among higher hydrophobicity classes especially in the top 15 cm of the profile, below which spekboom takes dominance as the overall hydrophobicity declines, as illustrated by an increase in the proportion of hydrophilic soils. Further illustrations of notable differences with depth are depicted in Fig. 2.13.

Table 2.3 Legend illustrating allocation of hydrophobicity classes according to WDPT time, adapted from Bisdom et al. (1993).

WDPT time (s)	WDPT class
< 10	Non-repellent
10-60	Slightly repellent
60-600	Strongly repellent
600-3600	Severely repellent
> 3600	Extremely repellent

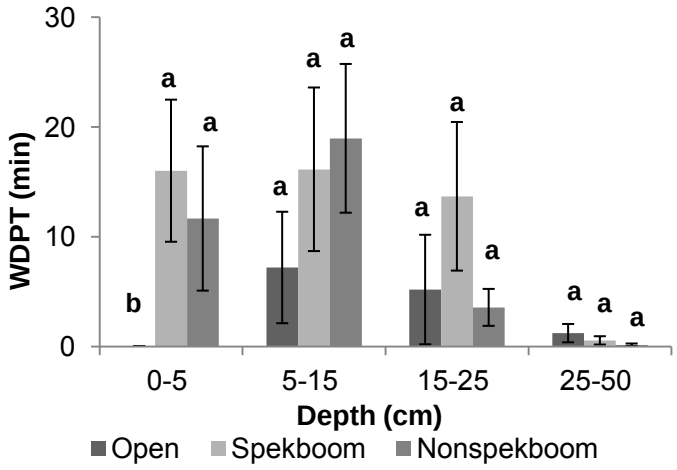

Fig. 2.12 Persistence and distribution of hydrophobicity with depth between sites.

Note: Error bars represent standard error, and alphabetic letters denote statistical differences between sites at specific depth according to the least significant difference (lsd) of means at α = 0.05. Similar letters indicate lack of significant differences.

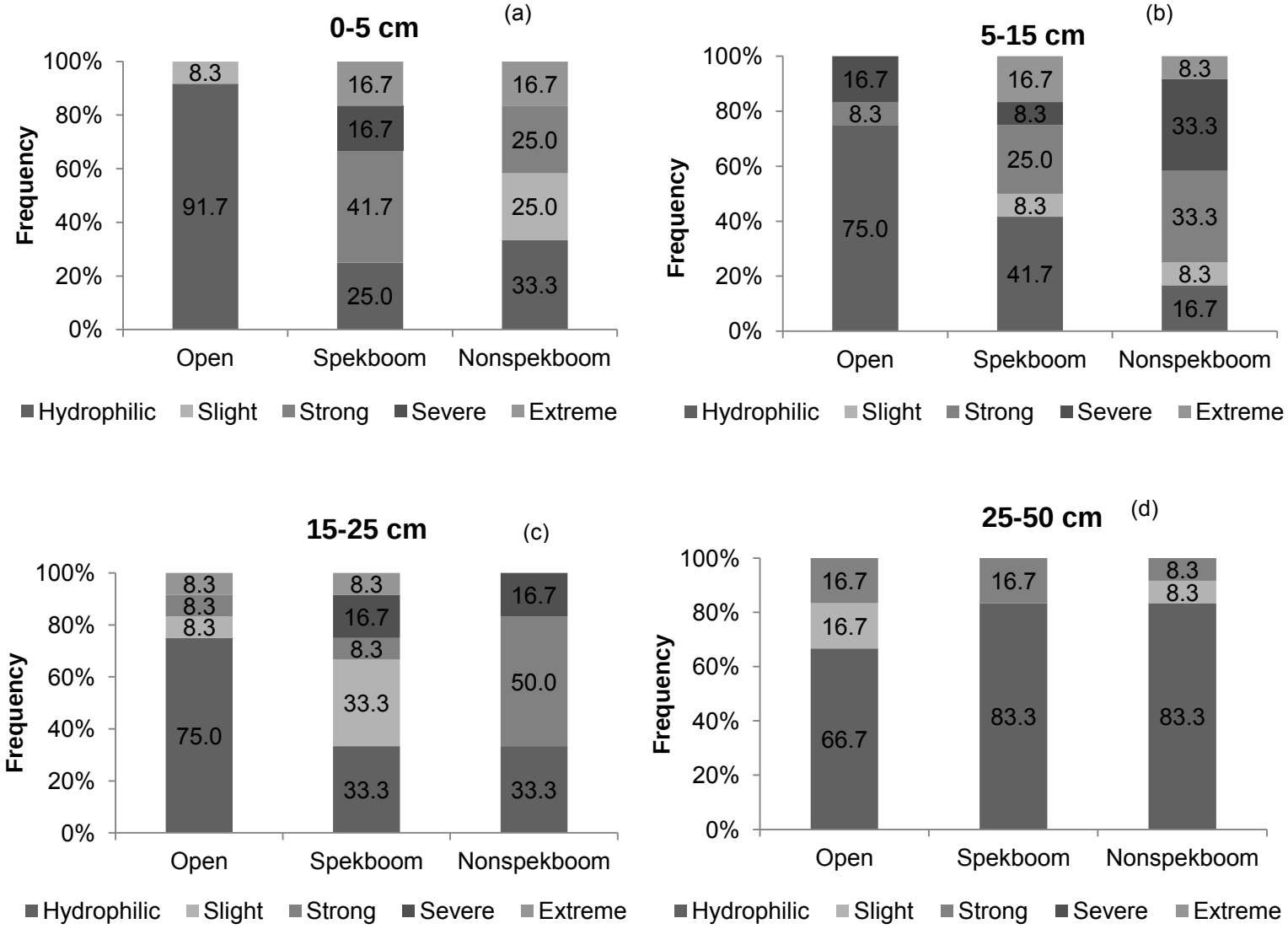

Fig. 2.13 Occurrence of soil hydrophobicity and its relative frequency with depth.

The observed patterns and widespread nature of hydrophobicity in the thicket is surprisingly high considering the low susceptibility often associated with calcareous soils. These results demonstrate a contrast to the widely acknowledged concept that acidic soils are more likely to develop hydrophobicity than alkaline soil because of the close association of hydrophobicity with the humin component of SOM (Bisdom et al., 1993, Roberts and Carbon, 1971). The humin fraction is insoluble under acidic conditions, but soluble, and hence prone to leaching under alkaline conditions (Mataix-Solera and Doerr, 2004, Wallis and Horne, 1992). In this study however, a significant interaction was found between site and depth; significant correlations were evident for open sites at 5-15 cm (r^2 = 0.60), as well as, nonspekboom sites at 15-25 (r^2 = 0.51) and 25-50 cm (r^2 = 0.69), as depicted in Fig. 2.14 here below.

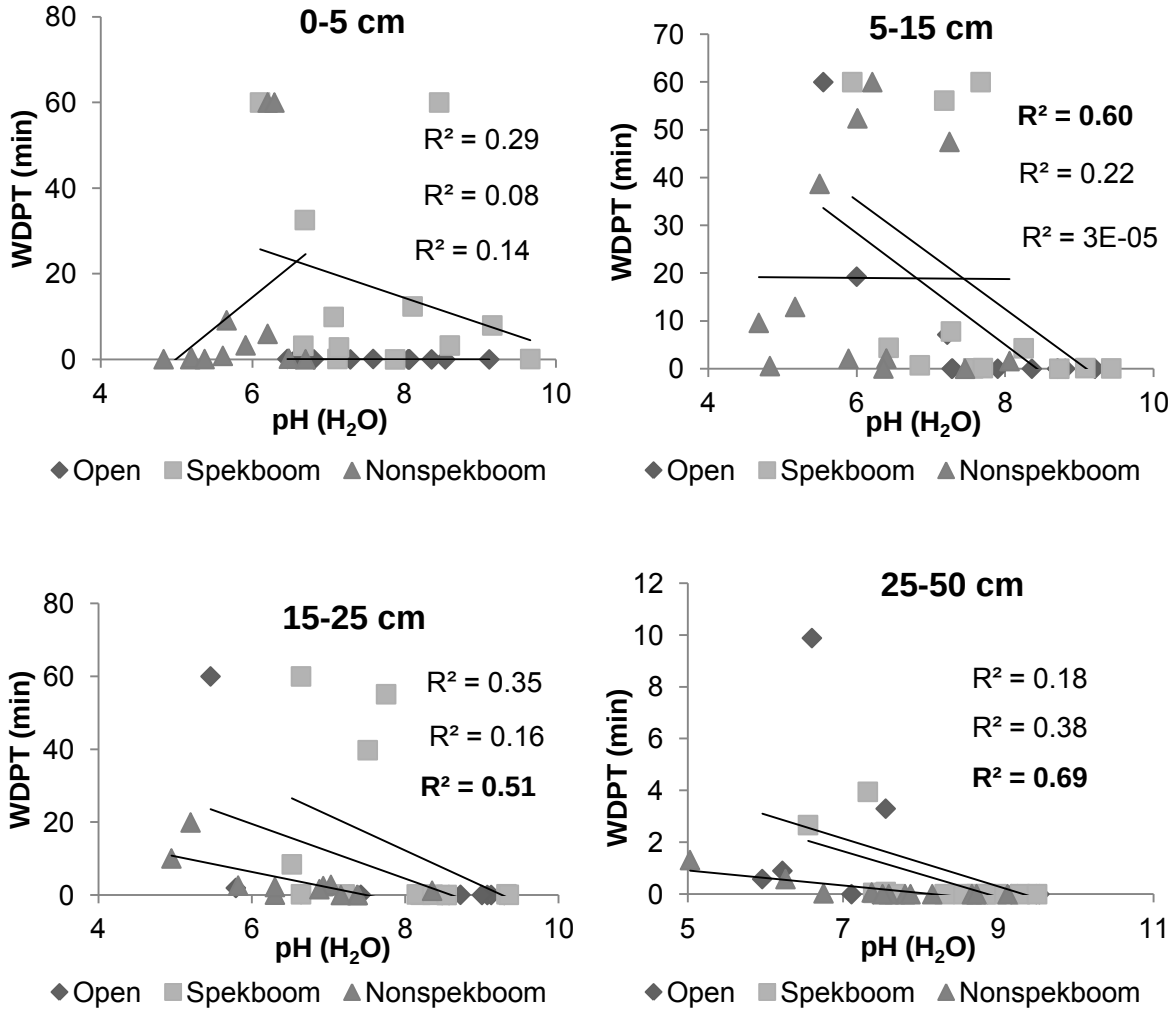

Fig. 2.14 The effect of pH on the degree of hydrophobicity with depth.

Note: r^2-values are arranged in a vertical order of open, spekboom, and nonspekboom.

Lack of response with regards to hydrophobicity of spekboom soils to pH dynamics suggests a potential likelihood for another mechanism(s) with a stronger influence over hydrophobicity. Soil organic matter is well known as an essential source and sorbent of hydrophobic organic compounds in soils, and can thus affect transport and bioavailability of hydrophobic substances (Xing and Pignatello, 1998). In its naturally dense intact state, the thicket soils tend to be water repellent, with a large organic C reserve. Reduced hydrophobicity following transformation is possibly linked to the marked losses in organic C observed in Fig. 2.5 (b). This compelled further investigation into the relations of hydrophobicity and organic C distribution with depth among the different sites.

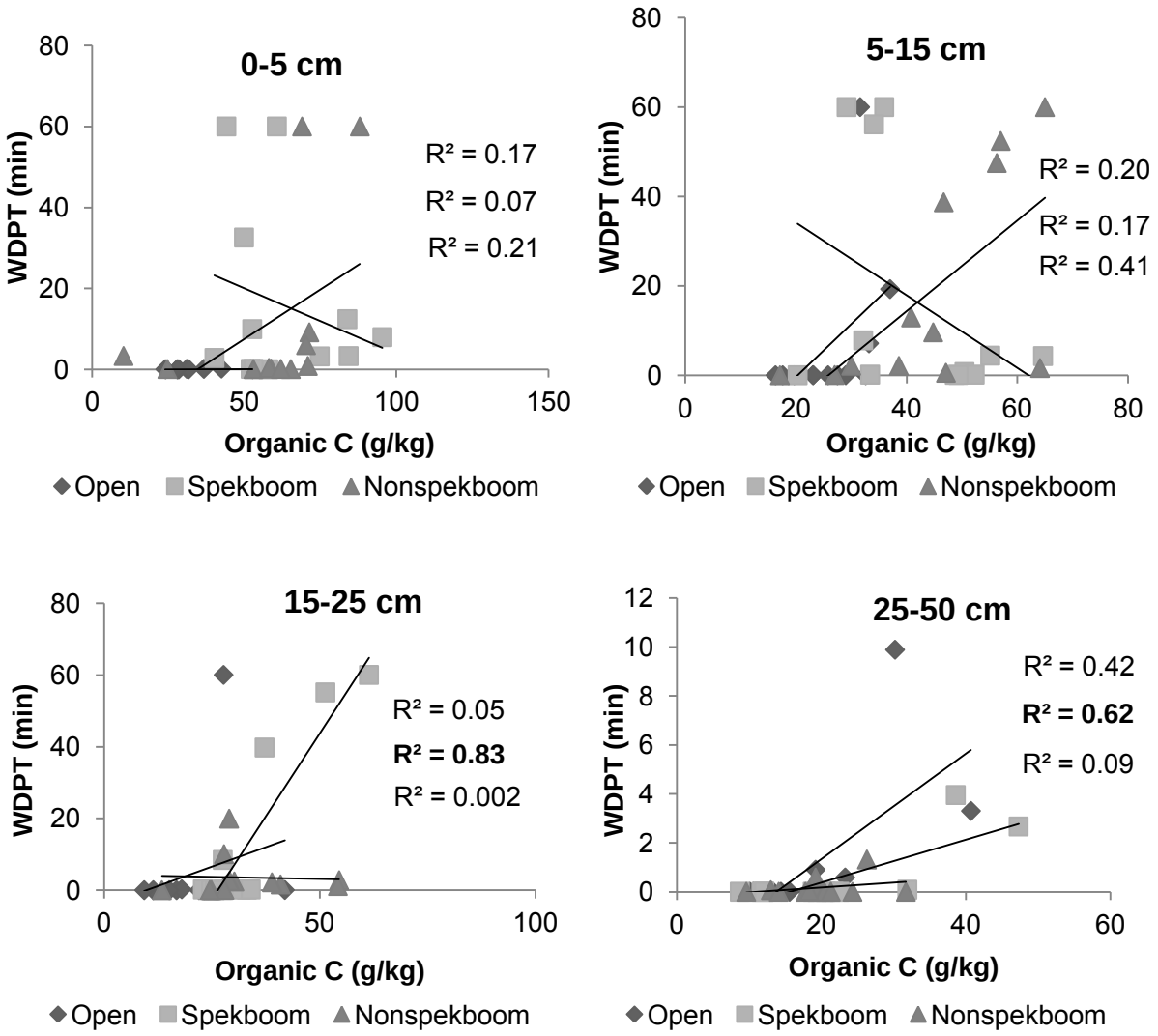

Fig. 2.15 The influence of organic C on the persistence of hydrophobicity at various depths among the sites.

Note: r^2-values are arranged in a vertical order of open, spekboom, and nonspekboom.

Spekboom soils reflected stronger correlations of hydrophobicity with organic C (Fig. 2.15), especially at 15-25 cm (r^2 = 0.83) and 25-50 cm (r^2 = 0.62), than previously witnessed with pH. Open and nonspekboom soils did not show any significant response in hydrophobicity in the direction of organic C (r^2 < 0.50). It appears as though different mechanism(s) influence soil hydrophobicity at the different sites, and this effect fluctuates with depth. For instance, lower pH conditions under nonspekboom species promote development of hydrophobicity below 15 cm, a similar effect was observed at 5-15 cm of open sites (Fig. 2.14). On the contrary, hydrophobicity of spekboom soils related more strongly to organic C concentration than pH; r^2 = 0.83 vs. 0.16 at 15-25 cm and r^2 = 0.62 vs. 0.38 at 25-50 cm for organic C and pH, respectively (Fig. 2.14 and Fig. 2.15).

Some researchers have attributed soil hydrophobicity to the hydrophobic nature of fine particles of the soil matrix e.g. Bisdom et al. (1993), and some to low pH e.g. Roberts and Carbon (1971). In this study however, strong correlations between hydrophobicity and organic C content (especially under spekboom sites) suggest that soil hydrophobicity is most likely due to organic matter coatings formed around individual soil particles during aggregate formation. McGhie and Postner (1981) demonstrated how relatively small amounts of fine, organic hydrophobic material can induce hydrophobicity in a sample. Fire-induced hydrophobicity can be ruled out, given the natural exclusion of fire in the thicket in its intact state, attributable to the succulent non-combustible nature of spekboom. This further corroborates a close association between hydrophobicity and the nature (possibly chemistry as well) of the vegetation occurring in the thicket, through the release of natural hydrophobic compounds e.g. organic acids, resins, waxes, and/or aromatic oils (Doerr et al., 1998) by the roots and plant tissues (Wallis and Horne, 1992).

The contemporary state of transformation in the open area landscapes indicates that the vegetation structure has completely lost its natural succulence through disappearance of spekboom species, and as a consequence open sites are now prone to wildfires. Occurrence of fire would increase the degree of hydrophobicity in these soils, which could have serious adverse effects on water infiltration and storage. Hydrophobic compounds are of organic nature (Mataix-Solera and Doerr, 2004), and the litter layer is a critical source of these hydrophobic substances. This

is most probably attributable to the reduced capacity of open area sites to produce litter and to replenish hydrophobic compounds. In dry regions such as this study area, hydrophobicity may aid in water conservation by minimizing moisture losses through evaporation.

2.3.4 Bulk density and C stocks

Soil carbon stocks are a measure of mean carbon content of soil mass per given area, thus it is necessary that the bulk density be computed for accurate measure of C stocks. The degraded (grass-vegetated) “open” sites displayed significantly higher soil bulk density in the top 40 cm, relative to intact thicket sites beneath spekboom canopies (Table 2.4).

Table 2.4 Comparison of soil bulk density with depth between degraded (open) and intact (spekboom) sites.

Depth (cm)	Open Bulk density (kg/m^3)	Spekboom Bulk density (kg/m^3)	P-value
0-20	1482.1 ***a***	994.5 ***b***	0.0001
20-40	1540.8 ***a***	1086.6 ***b***	0.0009

Note: Alphabetic letters denote statistical differences between sites at specific depth according to the least significant difference (lsd) of means at α = 0.05. Similar letters indicate lack of significant differences.

Thicket restoration movement intends to re-establish spekboom cuttings in the open (degraded) sites (Mills and cowling, 2010); thus, C stocks (t ha^{-1}) comparison between open vs. spekboom sites is of utmost importance in this study. Soil bulk density had a significant effect on C stocks; despite the observed differences in C concentration and distribution across the transect line; mean C stocks (total, organic, and inorganic) to a depth of 50 cm did not differ between degraded and intact sites (Fig. 2.16). The variation in both organic and inorganic C between open and spekboom sites is explicated by bulk density variation, corroborated by lack of differences when bulk density is incorporated.

The overall effect of degradation on C stocks was found to be minimal under current conditions; 355.8 ± 41.3 vs. 374.1 ± 35.3 t ha^{-1} cumulative total C to a depth of 50 cm under open and spekboom sites, respectively. Relative differences in both organic (306.0 ± 35.2 vs. 324.9 ± 34.2 t ha^{-1}) and inorganic (52.6 ± 18.1 vs. 49.8 ± 10.6 t ha^{-1}) C stocks were not statistically different at α = 0.05 in the top 50 cm between open and spekboom sites, respectively (Fig. 2.16 b and c).

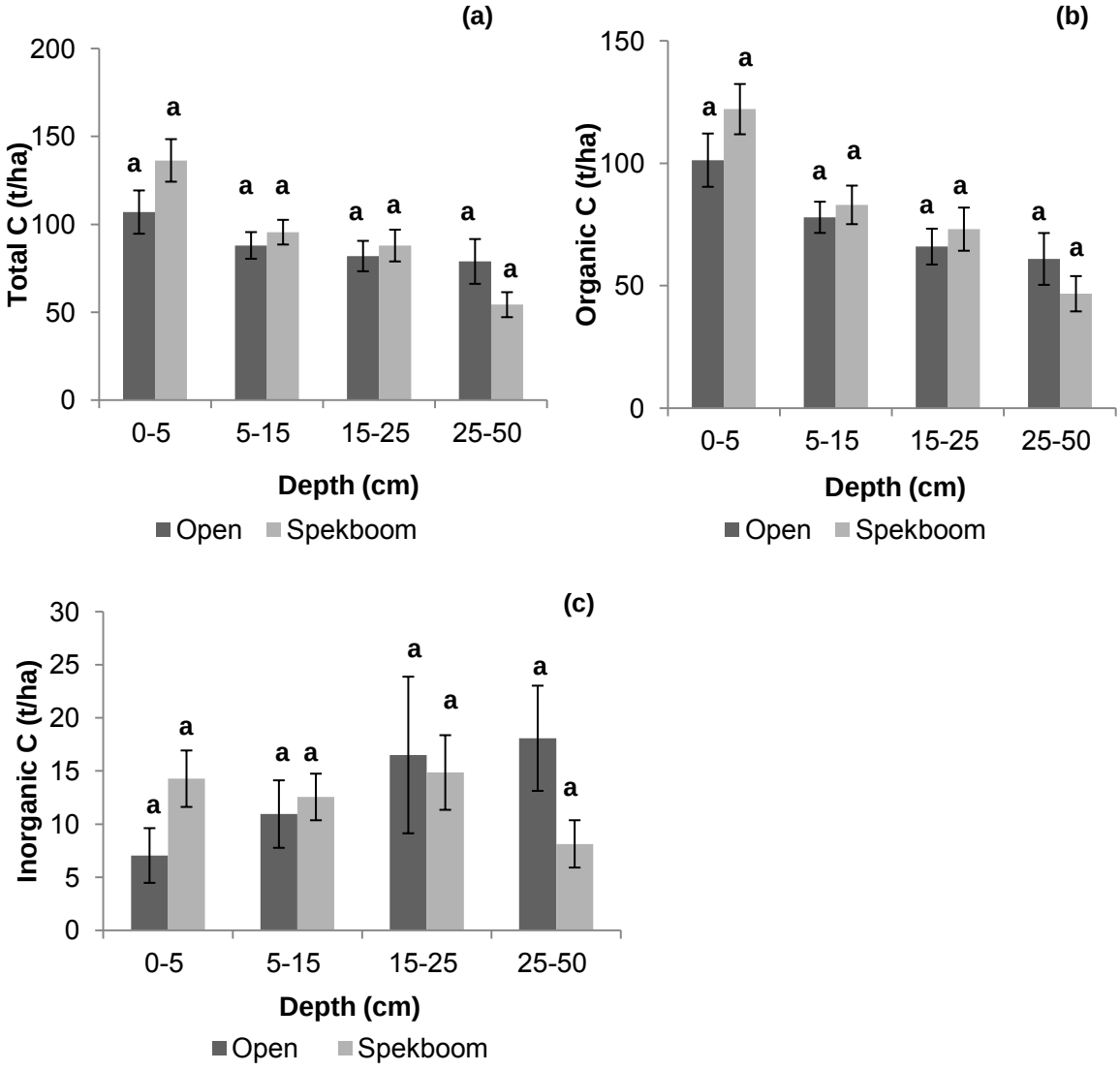

Fig. 2.16 Distribution of total (a), organic (b), and inorganic (c) carbon stocks to a depth of 50 cm between degraded (open) and intact (spekboom) sites.

Note: Error bars represent standard error, and alphabetic letters denote statistical differences between sites at specific depth according to the least significant difference (lsd) of means at α = 0.05. Similar letters indicate lack of significant differences.

The intact thicket total C stocks estimated in this study (on average 319.8 ± 28.2 t C ha^{-1} at 0-25 cm) are exceedingly higher compared to those previously reported by Mills and Cowling (2010) in the Baviaans Spekboom Thicket (BST); *ca*. 52 ± 5 t ha^{-1} of total C to a depth of 25 cm. However the degraded landscapes of this study were not as severely degraded as the BST plots judging from the total C stocks in the open area sites (25 cm); 18 ± 1 vs. 276.9 ± 28.6 t ha^{-1} for the BST plot and current study, respectively. In light of other studies; Jobbagy and Jackson (2000) estimated *ca*. 174 and 158 t ha^{-1} for temperate vs. tropical deciduous forests, and 186 t ha^{-1} for evergreen tropical forests vs. only 62 t ha^{-1} SOC for desert ecosystems, to a depth of 1 meter (Table 2.5). In this study however, a mean of 324.9 t ha^{-1} SOC under intact spekboom thicket vs. 306.0 t ha^{-1} in the degraded (open) thicket landscapes was estimated, over a depth of 50 cm. Furthermore, organic C stocks accumulating in soils of this semi-arid thicket, over a depth of 25 cm (278.2 ± 27.0 t ha^{-1} under intact vs. 245.1 ± 24.6 t ha^{-1} in the degraded sites) are far greater than those estimated by Batjes (2011) for moist tropical (50 t ha^{-1}), as well as wet and montane tropical (61 t ha^{-1}) climatic zones at 30 cm depth.

Table 2.5 Global soil organic carbon distribution according to biome (Jobbagy and Jackson, 2000)

Biome†	No. samples‡	Land area† (10^{12} m^2)	SOC content (kg/m^2) by depth (m) 0–3	0–1	1–2	2–3
Boreal forest	648, 118	12	12.5 (8.8)	9.3 (7.0)	2.4 (2.9)	0.8 (2.1)
Crops	1271, 837	14	17.7 (12.9)	11.2 (7.7)	3.8 (4.2)	2.7 (3.4)
Deserts	285, 161	18	11.5 (8.2)	6.2 (3.4)	2.9 (3.2)	2.4 (3.4)
Sclerophyllous shrubs	62, 38	8.5	14.6 (7.9)	8.9 (3.9)	3.3 (3.0)	2.4 (2.0)
Temperate deciduous forest	60, 41	7	22.8 (13.6)	17.4 (10.8)	3.3 (3.7)	2.1 (2.4)
Temperate evergreen forest	123, 85	5	20.4 (12.0)	14.5 (8.4)	3.6 (3.7)	2.3 (2.6)
Temperate grassland	121, 83	9	19.1 (10.9)	11.7 (6.6)	4.2 (3.7)	3.2 (2.8)
Tropical deciduous forest	29, 24	7.5	29.1 (14.6)	15.8 (9.2)	7.4 (4.4)	5.8 (3.3)
Tropical evergreen forest	36, 30	17	27.9 (8.9)	18.6 (10.4)	5.4 (3.1)	3.9 (2.2)
Tropical grassland/savanna	35, 27	15	23.0 (19.9)	13.2 (8.7)	5.5 (6.1)	4.2 (5.1)
Tundra	51, 12	8	18.0 (15.2)	14.2 (14.1)	2.4 (3.1)	1.4 (2.1)
Global average	2721, 1456	121				

† Biome classification and land area are based on Whittaker (1975) and Jackson et al. (1997).

2.4 CONCLUSIONS

Spekboom soils were found to contain significantly higher concentrations of exchangeable bases; Mg, Na, and K in particular, and subsequently higher soil EC and pH values, relative to other sites. This appeared to be attributed to the nutrient composition of the source plant tissue, as indicated by litter and foliage nutrient composition of each vegetation, which demonstrated a gradually increased in exchangeable Mg, Na, and K in the direction of spekboom > nonspekboom > open. In a similar fashion as exchangeable bases, water-soluble Na and K were significantly higher under spekboom than nonspekboom sites, which explains the high pH values encountered underneath spekboom canopies, relative to lower pH values displayed by nonspekboom soils.

Although certain soil chemical parameters varied appreciably within the intact thicket (i.e. between spekboom and nonspekboom), this did not appear to result in any significant effect on the SOC content and hydrophobicity. This suggests that the thicket's ability to sequester carbon in the soil is not species-specific, i.e. it is not solely reliant on spekboom species, but rather a holistic ecosystem functioning, provided that animal grazing is excluded or kept at minimal intensities. On the contrary, remarkable organic C losses were evident in the open sites; this amounted up to 46 % decline in the top 0-5 cm (34.0 g C kg^{-1}), relative to spekboom (62.4 g C kg^{-1}). In addition to SOC, substantial amounts (2-9 g C kg^{-1}) of inorganic soil C were detected on most soils (most likely derived from *in situ* weathering), and were not affected by the soil chemistry or vegetation.

The aboveground differences illustrated by the fence-line contrasts and amounts of litter were reflected in belowground soil chemistry (pH, EC, SOC etc.), physical structure (root density and aggregate stability), and hydrophobic behavior of soils. These findings support the hypothesis that the climatic constraints on SOC distribution commonly associated with semi-arid regions is somewhat eclipsed by the effects of the native vegetation cover occurring in the thicket. These results suggest that changes in vegetation species composition associated with the grazing-induced transformation of the thicket, has a significant effect on nutrient returns to soil, indicated by lower exchangeable bases and organic C contents. The degree of hydrophobicity was strongly influenced by the relative amounts of organic C for

spekboom sites, whereas pH had a considerable effect on open and nonspekboom sites. In spite of marked losses of SOC observed under open sites, the remaining quantities of SOC are relatively large for a semi-arid “savanna” ecosystem. Furthermore, there were no significant differences in both organic and inorganic C stocks of the degraded and intact sites; this is mainly due to higher bulk density of the soils from open sites. This most probably suggests a considerable degree of resilience maintained in the open sites. The relative stability of the organic C contained in these soils will be investigated in the following chapter.

CHAPTER THREE

CARBON STABILITY MECHANISMS IN SPEKBOOM THICKET SOILS

3.1 INTRODUCTION

Stabilization of organic matter (OM) in soils merely refers to its preservation against breakdown. Its preservation however is not permanent and over time its breakdown (decomposition) by soil organisms becomes inevitable. Several ecosystem mechanisms have been postulated according to which ecosystems naturally protect OM, these can be broadly grouped into three fundamental mechanisms; (i) biochemical recalcitrance, (ii) soil aggregation, and (iii) organo-mineral associations (Mikutta et al., 2006, von Lützow et al., 2006, Christensen, 1996, Sollins et al., 1996, von Lützow et al., 2007). These mechanisms as well as physicochemical processes such as hydrophobicity, ligand exchange, cation bridging etc. are actively involved in C stabilization, discussed in great detail in Chapter 2.

Intimate associations of soil organic carbon (SOC) with soil mineral surfaces have been widely researched in the past decade e.g. Torn et al. (1997). Incubation experiments by (Miltner and Zech, 1998) have verified the decline in decomposition rates of organic matter that is associated with mineral surfaces. Chemical properties of mineral surfaces and structural properties of the organic matter involved determine the degree of stability and turnover time (Mikutta et al. 2009), with aliphatic and aromatic structures being the most stable with a longer turnover time (Derenne and Largeau, 2001, Marín-Spiotta et al., 2008, Mikutta et al., 2007, Ostertag et al., 2008). Poorly crystalline soil minerals especially have a larger capacity to chemically stabilize OM through sorption reactions due to their large specific surface area (Bracewell et al., 1970, Schwermann et al., 1986), thereby retarding microbial degradation (Huang et al., 2005). In addition to stabilization on mineral surfaces, chemical transformation of organic matter (humification) often play an important role in the synthesis of new recalcitrant compounds, and many studies e.g. Baldock et al. (1992), Baldock and Preston (1995), Martínez et al. (2007) etc. have confirmed that the presence of aliphatic and aromatic carbon can slow down litter decomposition rates.

Soil organic matter (SOM) consists of various functional pools, defined by their characteristic turnover times and pool sizes (Christensen, 1992). Physical and chemical fractionation techniques are commonly used in soil studies to isolate the different organic matter pools, often to quantify these pools and examine their relative stability. Soil organic matter density fractionation provides an indispensable technique to separate the different SOM pools in order to isolate the free (uncomplexed) OM from the stable pools occluded within macro- and micro-aggregates (von Lützow et al., 2006) and bound to soil minerals. This chapter investigates the research question, how and why (i.e. mechanisms) such large amounts of organic C accumulate in a semi-arid region beyond the results global models of SOC distribution.

Two major SOM functional pools are of great importance in soil carbon stabilization studies; the uncomplexed particulate organic matter (POM) and the stable mineral-bound organic matter (MOM). Isolation of these pools from whole soils facilitates quantification of organic carbon contained in these pools and their relative contribution to total organic carbon, as well as advanced study of each individual pool with spectroscopic techniques such as ^{13}C NMR and FT-IR to evaluate its composition relating to its stability. The POM pool represents the labile, mineral-free fraction of OM that is most sensitive to management outcomes than bulk SOM (Baldock and Nelson, 2000), whereas the MOM pool refers to OM that is stabilized on surfaces of clay minerals and oxides via sorption reactions of ligand exchange (Gu et al., 1994, Kaiser and Guggenberger, 2003), or cation bridging (Oades, 1988). In this study, the stable MOM fraction is inclusive of the intra-aggregate OM that is occluded within soil aggregates and physically protected from the decomposing community by exclusion (von Lützow et al., 2006). Poorly crystalline Al and Fe oxy-hydroxides play a major role in stabilization of this particular fraction attributable to their large surface area available for OM adsorption (Mikutta et al., 2006).

Previous research in other semi-arid ecosystems e.g. Vaieretti et al. (2005), Martínez et al. (2007), and Rasmussen and White (2010) have evaluated the controls of vegetation on soil organic matter quality and soil organic carbon (SOC) dynamics. Rasmussen and White (2010) found significant differences in organic C distribution between pools, with least concentrations under grassy sites compared to woody

(mesquite and creosote) vegetation. They found only slightly greater proportion of bulk organic C to be mineral-bound (37-62 %) relative to particulate organic C (26-40 %) on average, with little evidence for aggregation. Additionally they found all vegetation types to be thermally labile, indicated by high (>1) exothermic indices (Exo_1/Exo_2).

In general the role of carbon stabilization mechanisms in arid and semi-arid ecosystems is often disregarded, due to the small amounts of carbon commonly encountered in these environments, attributed to prevailing climatic conditions. However, vegetation control can alter the anticipated regimes, such as in this particular case in the spekboom thicket. Some studies actually have estimated organic carbon content of various semi-arid regions under different land uses e.g. pastures, cultivated lands, forests etc. as well as vegetation types e.g. grasslands, woodlands, and forests. Guo and Gifford (2002) carried out a meta-analysis study where they evaluated the effects of land use change on soil carbon content. Most literature studies in the past only evaluated the overall effects of ecosystem changes on the total organic carbon e.g. McCulley et al. (2004), some proceeded only as far as quantifying the particulate organic carbon content e.g. Golchin et al. (1994) and Sohi et al. (2001). Very few authors e.g. Rasmussen and White (2010), Grünewald et al. (2006), and Steffens et al. (2009, 2011) have gone further to estimate the stable SOC fraction and the extent of its relative stability in semi-arid ecosystems.

Previous research carried out on spekboom thickets by Mills et al. (2005) and Mills and Cowling (2010) have recognized the phenomenal wealth of the spekboom thicket biomes, with regards to their capacity to accumulate large amounts of carbon in their soils. However, there has been little targeted research on how much of this carbon is organic, and also the extent of its stability given the land use change and the ever changing environment.

The main objective of this experimental chapter is to advance relevant knowledge pertaining to large amounts of carbon accumulating in the semi-arid spekboom thicket soils, contrasting to other similar ecosystems. The task at hand was to quantify the stable mineral-bound fraction of the organic carbon, which represents the refractory fraction that is not easily destructed by natural ecosystem processes. This fraction constitutes a core parameter pertaining to soil carbon sequestration and

conservation, thus comprehensive knowledge of how it is stabilized (mechanisms) and the extent of its stability is critical as it reflects the capacity of terrestrial ecosystems to sequestrate organic carbon in the soil. This entailed a detailed investigation of the relative stability of the SOC under intact (spekboom and nonspekboom) and degraded (open) landscapes of the thicket. A further aim was to elucidate the possible chemical and physical mechanisms responsible for the stabilization SOC in spekboom thicket soils. This was carried out by examining the relationships of the stable mineral-bound SOC fraction with selected soil properties such as metal oxide content, clay content, aggregate stability, and hydrophobicity in an attempt to elucidate the mechanism(s) accountable for SOC stability. Furthermore, we aimed to explore the chemical structural composition of the organic materials at the various stages of decomposition using spectroscopic techniques (FTIR and NMR spectroscopy). This was carried out to see whether the chemical nature of the organic materials could explain the recalcitrance of the SOC in the spekboom soils.

3.2 MATERIALS AND METHODS

Soils were collected from three sites; open, spekboom, and nonspekboom. Each site was replicated across 12 transects in the field, as described in detail in Chapter II (page 29). In soils, OM sorption onto mineral surfaces is primarily reliant on the clay size (< 2 µm) particles (Kögel-Knabner et al., 2008). For this reason, subsoil (5-15 cm) samples with sufficiently high clay content were used for this meticulous study, with the premise that the MOC contained in these soils will reflect the sorption capacities of minerals and metal oxides. The selected sample depth (5-15 cm) also ensures that the litter material is not incorporated in the sample, which would in turn overestimate the POM pool size. For the purpose of this study (chapter), various other analyses (mineral composition, particle size and texture analysis, and aggregate stability) were also performed specifically at 5-15 cm for correlation analysis. The twelve fence-line transects were treated as field replicates of the three sites (open, spekboom, and nonspekboom) at 5-15 cm, thus 12 samples per site.

3.2.1 Mineralogical composition

The mineralogical composition of the soils was examined by clay separation and X-Ray diffraction (XRD) analysis of K and Mg saturated samples (Whittig and Allardice, 1986) at 45 kV and 30 mA, using the Cu Kα radiation. The analysis was carried out on three soil samples randomly selected from various transects. Further mineralogical parameters such as Fe and Al concentrations were determined by selective extractions, exclusively for the spekboom site (i.e. 12 samples) in order to seek correlations to the MOC. Total Fe (crystalline & poorly crystalline) and poorly crystalline Al concentrations were determined according to the citrate-dithionite-bicarbonate (CDB) extraction (Jackson et al., 1986). After sample pre-treatment with 1 *M* pH 5.5 ammonium acetate, poorly crystalline Fe was extracted with acid ammonium oxalate (Tamm's reagent) under dark conditions (Loeppert and Inskeep, 1996).

3.2.2 Aggregate stability

The wet sieving technique was employed to examine the relative stability of soil aggregates between the three sites according to Kemper and Rosenau (1986). The technique operates on the principle that unstable aggregates will break down more voluntarily than stable aggregates when submerged in water. Only macro-aggregates (0.25–2 mm) were investigated in this study. The analysis was carried out on duplicated samples under laboratory conditions using the Eijkelkamp E-365-08.13 wet sieving apparatus (Eijkelkamp Agrisearch Equipment, Netherlands).

3.2.3 Fractionation of SOM pools

Trial study

Initially, a series of trial studies were conducted to test the reproducibility and suitability of the proposed methodologies for SOM fractionation specifically on our soils. These trials were carried out on duplicate representative samples for each of the three sites (open area, spekboom, and nonspekboom) from the same fence-line transect in order to minimize spatial variation. The trial entailed a quantitative comparison of SOM pool sizes, as well as organic C concentration and overall contribution of each pool towards bulk SOC between sites. Samples with high total organic C contents were preferably selected.

The first trial entailed sequential isolation of bulk SOM into free and occluded particulate OM pools using physical fractionation techniques with density separation and ultrasonification, respectively. Several literature studies e.g. (Sohi et al., 2001, Christensen, 1996, Golchin et al., 1994) recommend density separation with sodium Iodide (NaI) or sodium polytungstate (SPT) solution at 1.60 and 1.80 g cm^{-3}, with 1500 J g^{-1} soil sonification for efficient recovery of free and intra-aggregate fractions respectively. Consequently, 15 g of soil with 90 mL of 1.80 g cm^{-3} NaI solution was used, followed by centrifugation at 8000*g* (*ca*. 10 000 rpm) after gently shaking the suspensions by hand, as recommended by Sohi et al. (2001). The light free POM was carefully transferred into a Millipore filtration funnel fitted with a 0.8 µm membrane filter and filtered under vacuum pressure. The soils had a large POM pool, and so the centrifugation – filtering procedure was repeated 3-4 times for each sample to quantitatively recover all POM. Thereafter, the isolated pools were thoroughly rinsed through the filter with distilled water under vacuum, and allowed to air-dry before subsequent analysis.

The results however depicted several discrepancies noted in weights of isolated OM pools of the same sample. This was most likely due to the tendency of NaI to become oily and sticky at such high density and thus adhere soil particles onto the organic matter, and making it impossible to dry the samples afterwards. Another experimental difficulty encountered was that low density minerals tend to float along with the organic matter during separation at such high solution density. Both of these factors may have caused confounding effects on the accuracy of intra-aggregate POM by overestimating the OM pool sizes due to inclusion of non-organic (mineral) material.

In an attempt to escape the aforementioned difficulties, a second trial was carried out on the same samples used in the first trial, though a lower NaI solution density and volume (30 mL 1.6 g cm^{-3} NaI) with a smaller sample (5 g soil) was used instead, whilst maintaining a similar 1:5 soil to solution ratio. Lower solution density of 1.6 g cm^{-3} is also acceptable and commonly used in OM density fractionation studies e.g. Golchin et al. (1994), Sollins et al. (1999), and Grünewald et al. (2006) since OM density is generally less or equal to 1.5 g cm^{-3}. Similar centrifuging and filtering conditions were used as in the first trial study. The method reproducibility was found

to be satisfactory and the results displayed reduced variation between duplicates when a smaller sample size and reduced solution density was used.

After separation of the free POM, the heavy residual sample was washed into a 150 mL beaker with NaI solution and adjusted to a suspension volume of 90 mL. The suspensions were then sonified with an Omniruptor 400 ultrasonic homogenizer fitted with a 9 mm probe tip, to disrupt the aggregates and liberate the intra-aggregate POM occluded therein. A calorimetric approach was used to calibrate the instrument using distilled water (260.3 g) at various power settings (Schmidt et al., 1999), as the amount of energy produced by the sonifier probe cannot be measured directly. The actual power output was then calculated according to the following equation:

$$P = m_w c_w \Delta T / t + H \qquad (1)$$

Where P is the power output (W), m_w is the mass of water (g), c_w is the specific heat capacity of water (4.18 J kg^{-1} K^{-1}), ΔT is the difference in temperature (K), t is the duration of sonification, and H is the energy in J s^{-1} lost by conduction. The duration of sonification required to attain 1500 J g^{-1} soil was calculated to be 3 minutes and 2 seconds at 41.16 W (50%) instrumental power for a 5 g soil sample.

The sample residue remaining after all (free and intra-aggregate) POM had been removed, was dialyzed in a Pierce (Perbio) SnakeSkin pleated dialysis tubing in a bucket of distilled water until the water tested free of salts with 0.1 *M* $AgNO_3$, then oven-dried at *ca*. 40 °C prior to chemical oxidation.

Optimized appropriate fractionation methodology

The subsequent trial studies provided valuable insight and experience which aided in the selection of appropriate methodology specifically suited for our soils. Mikutta et al. (2009) recommended omission of ultrasonic dispersion to avoid the risk of transferring some of the low density mineral-organic associations (MOAs) into the particulate fraction during isolation of intra-aggregate OM, since certain MOAs (e.g. Al-OM associations) typically exhibit low absolute densities of 1.7 g cm^{-3} (Mikutta et al., 2009, Kaiser and Guggenberger, 2007). Moreover, several literature studies have esesentially identified IPOM to be more resistant (stable) against decomposition, as suggested by narrower C:N ratios (Gregorich et al., 2006) and

chemical composition i.e. greater alkyl C/O-alkyl C ratios relative to free POM (Golchin et al., 1994, Sohi et al., 2001). It typically comprises a vast mixture of OM (from partly decomposed OM fragments, microbial and faunal secretions, dead roots, and even humified material) formed via multiple mechanisms (von Lützow et al., 2007), thus contain characteristically different functional pools in terms of stability and turnover rates. Therefore in this study, fractionation was carried out without ultrasonification in order to avoid overestimation of pool fractions and thus IPOM was considered stable and collectively merged with the mineral-bound fraction.

This detailed carbon stabilization study was conducted on 5-15 cm soils, sampled directly underneath tree canopies of spekboom and nonspekboom species, as well as open (grassy) sites. Suspensions of 30 ml of 1.6 g cm^{-3} NaI and 5 g of soil were agitated at 3000 rpm for 30 minutes on a horizontal shaker, then centrifuged for 30 minutes at 4500 rpm (Mikutta et al., 2009) to separate the light POM form heavy MOAs. The floating POM was isolated by carefully filtering the samples through a 0.8 µm membrane filter. The centrifugation-filtering procedure was repeated three times for each sample to quantitatively transfer all POM; thereafter, the isolated pools were thoroughly rinsed with distilled water and allowed to air-dry before subsequent analysis. The isolated individual POM and MOM pools were weighed on a digital micro-scale (OHAUS Analytical Plus) and analyzed for C and N by dry combustion (Nelson and Sommers, 1996) using the Varian elemental analyzer.

3.2.4 Spectroscopic and thermal analyses

Fourier-Transform Infrared (FT-IR) and ^{13}C NMR CPMAS spectroscopy techniques were used to characterize the chemical structural composition of selected organic material samples. Composite, litter and foliage samples, as well as, density fractions of the different sites were analyzed with FT-IR spectroscopy. Additionally, POM, MOM, and litter samples from a representative spekboom site were extensively analyzed with solution state, cross polarization, magic angle spinning (CP-MAS) ^{13}C NMR in order to quantitatively determine the relative quantities of the structural organic compounds associated with its recalcitrance, succulence.

3.2.4.1 FT-IR

The FT-IR technique operates on the principle that specific functional groups of organic molecules vibrate at a certain frequency regardless of which molecule they are attached to. The pellet method was used, with Potassium Bromide (KBr) as a reference material. Sample and KBr mixtures in 1:100 ratio respectively, were finely ground into powder and compressed in a 13 mm diameter pellet-pressing die, using a hydraulic press at *ca*. 8 kPa. The prepared pellets were that exposed to an Infrared beam and acquired 32 scans from 400 to 4000 cm^{-1} on a Thermo Scientific Nicolet 6700 spectrometer. Data processing was managed with Omnic software and Microsoft Excel (2007).

3.2.4.2 ^{13}C NMR

Solid state NMR spectra were acquired on a Varian spectrometer (VNMRS WB 500) using a 4 mm probe at 500 MHz static magnetic field strength. Adamantane was used as a reference material. The samples were spun at 12 kHz MAS using cross-polarization T3 HX MAS probe at ambient temperature with proton decoupling at a recycle delay of 4seconds and 1.5 ms contact time. Data processing handled with Spinworks, OriginLab v.8 and Microsoft Excel (2007).

3.3 RESULTS AND DISCUSSION

3.3.1 Mineralogy

The clay mineralogical composition was largely similar across the experimental sites; comprising of a mixture of mainly smectites/vermiculite, illite and some kaolinite and quartz (Fig. 3.1). X-ray diffraction analysis also detected traces of calcite. Crystalline Fe (αFe) constituted most of the sesquioxide fraction, thus low oxalate-extractable Fe (γFe/*t*Fe) < 20 % for most soils and γ(Fe+Al) < 10 g kg^{-1} for all samples (Table A3.2, appendix).

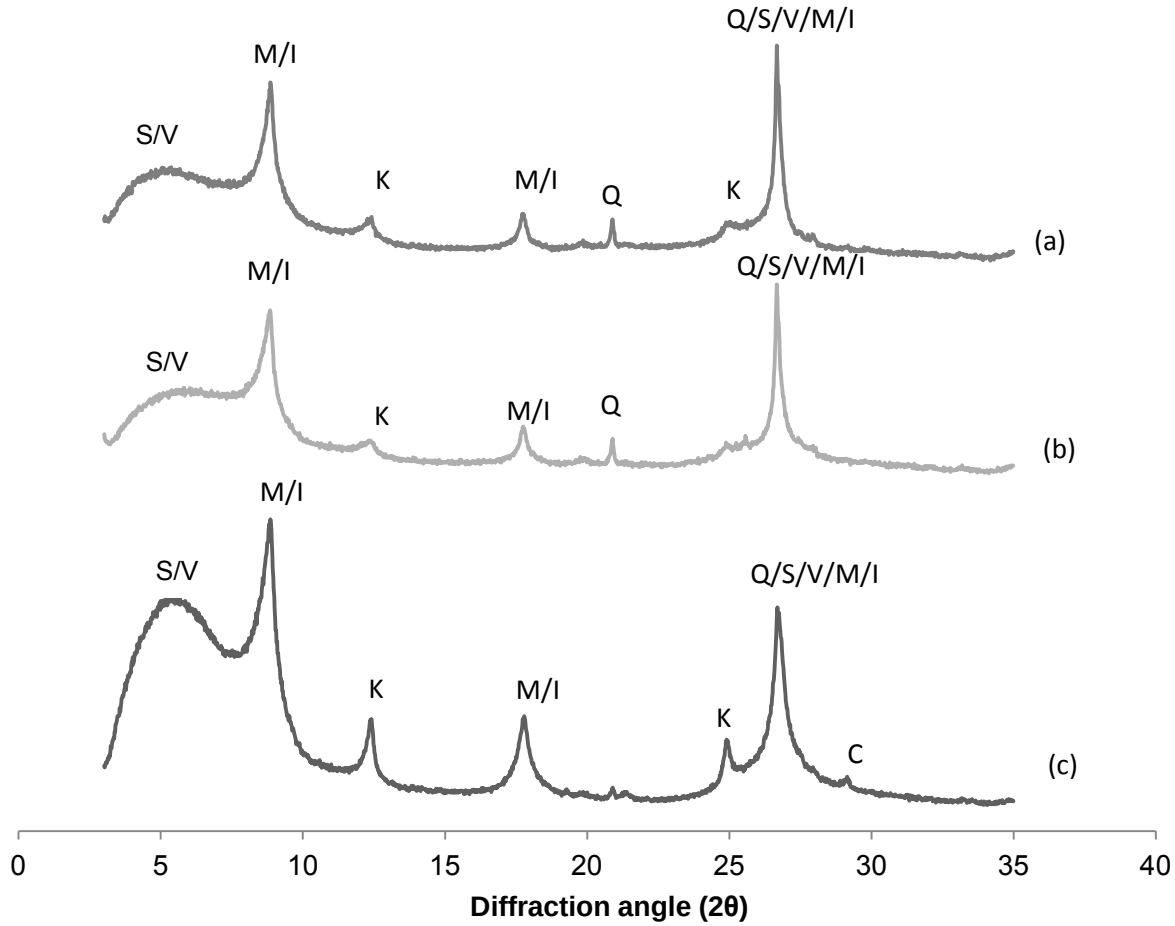

Fig. 3.1 Glycol-solvated, Mg-saturated X-Ray diffractograms of selected sites across the experimental plot. Sites (a), (b), and (c) correspond to shale, basalt, and limestone geology, respectively, according to Keyser (1997). S/V = smectite or vermiculite, M/I = Mica or Illite, K = Kaolinite, Q = quartz, and C = Calcite.

Table 3.1 Descriptive legend for the XRD-identified mineral peaks according to Whittig and Allardice (1986).

Diffraction angle (2θ)	d-spacing (Å)	Mineral
5.1-5.7	15.6-17.2	Smectite/Vermiculite
8.8	10.06	Mica/Illite
12.3	7.16	Kaolinite
17.6	4.99	Mica/Illite
20.8	4.27	Quartz
24.9	3.58	Kaolinite
26.7	3.34	Quartz/Smectites/Vermiculite/Mica/Illite
29.0	3.08	Calcite

3.3.2 Aggregate stability

The dynamics of intra-aggregate OM is closely interconnected to the lifetime of aggregates themselves (Besnard et al., 1996). Thicket transformation was found to impart notable effects on the stability of soil aggregates; macro-aggregates sub-sampled from open area soils were found to be less resistant to slaking in water, indicated by significantly lower aggregate stability values relative to intact thicket sites. Mean stability of aggregates averaged at 77 % for open areas, vs. 89.1 % and 88.7 % for spekboom and nonspekboom, respectively (Fig. 3. 2). The reduction in the proportion of stable aggregates is possibly related to high concentration of exchangeable Na in proportion to other cations (Fig. 2.10 chapter 2), which generally promote clay dispersion.

Furthermore, soil hydrophobicity is known to favour structural stability of aggregates (Hallett and Young, 1999) and consequently enhance the stability of intra-aggregate OM (Mataix-Solera and Doerr, 2004). Although, no significant relationships were found between aggregate stability and hydrophobicity in this study, it is possible that a combined effect with other soil properties e.g. organic C content may contribute to this phenomenon. Spekboom and nonspekboom sites on the other hand, displayed comparable trends in relative amounts of stable aggregates over a landscape scale.

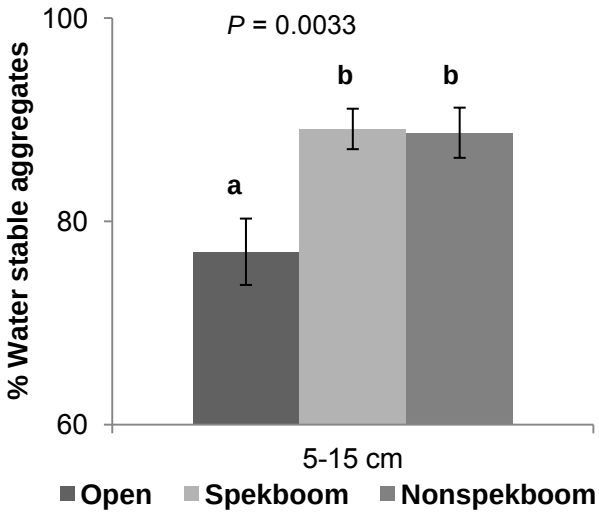

Fig. 3.2 Mean aggregate stability between sites at 5-15 cm.

Note: Error bars represent standard error, and alphabetic letters denote statistical differences between sites at specific depth according to the least significant difference (lsd) of means at α = 0.05.

3.3.3 Density fractions and organic C content

A meticulous investigation of SOC pools entailed characterization of the particulate and stable (mineral-bound) OM pools between the sites, specifically at 5-15 cm depth. The fractionatio was not replicated on a sample basis, the transects served as replicates instead, so as to meet the purpose of evaluating the effects of transformation and vegetation on SOC dynamics at corresponding soil depth (5-15 cm) and then use the acquired mean values to infer on landscape effects accordingly. The results reflected analogous trends to bulk SOC results between sites, open sites generally contained the least amounts of both POC and MOC.

Particulate organic carbon (POC) increased linearly with the weight of recovered particulate organic matter (POM) in the direction of nonspekboom > spekboom > open (Fig. 3.3 a and b). Particulate OM comprised *ca*. 2.4 ± 0.26, 3.7 ± 0.42, and 5.3 ± 0.60 % of soil dry weight (Fig. 3.3 a), and accounted for 7.0 ± 0.87, 9.3 ± 1.0, and 14.4 ± 1.32 g C kg^{-1} for open, spekboom, and nonspekboom respectively. Organic C contained in this fraction accounted for *ca*. 23-34 % of the total organic C (Fig. 3.3 c). The largest fraction (60-65 %) of organic carbon was found to be associated with the mineral fraction (MOC) for all sites, depicting similar distribution pattern as particulate and total organic carbon trends.

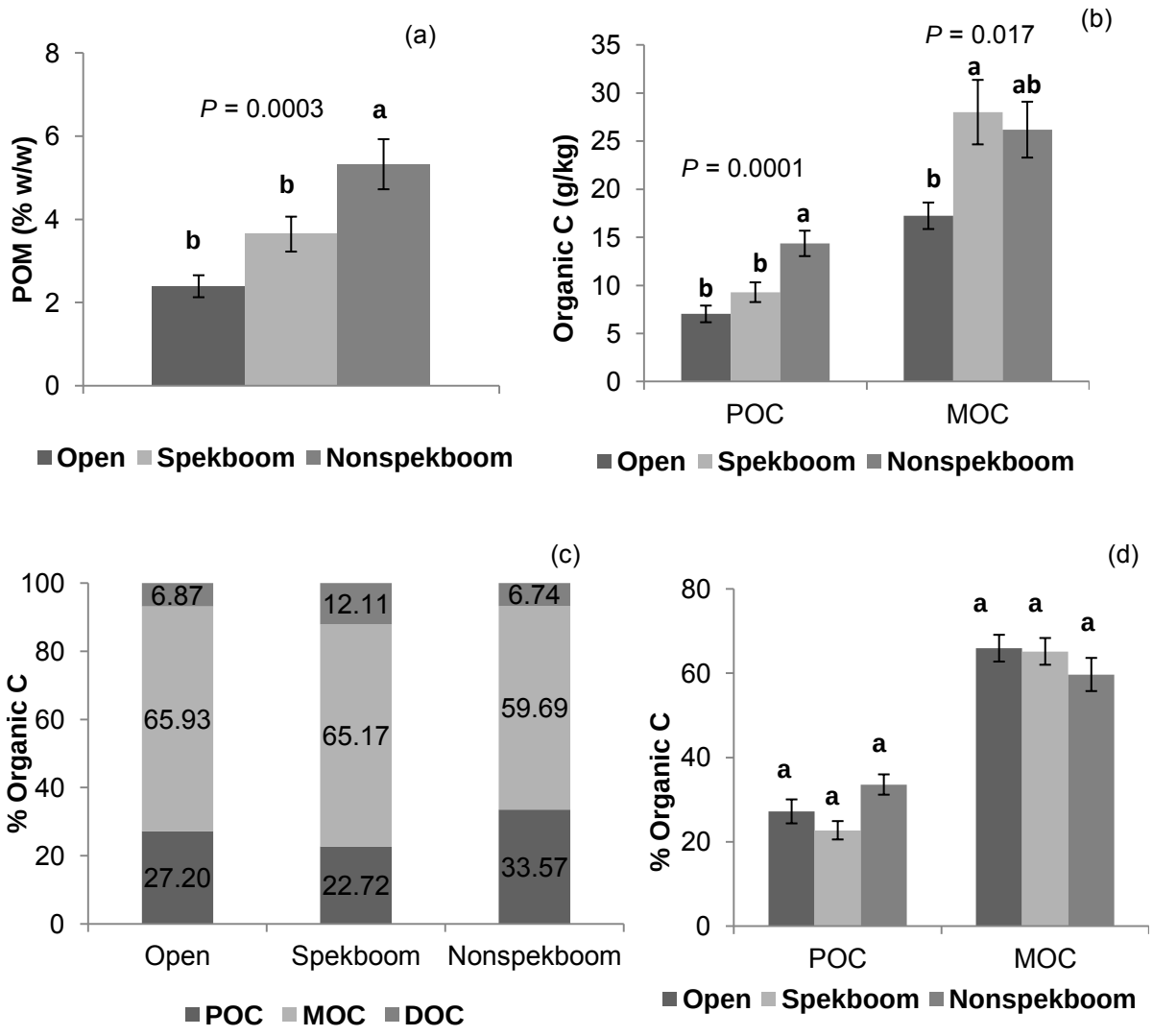

Fig. 3.3 (a) Recovered POM dry weight from bulk soil (% *w*/*w*), (b) organic C distribution between OM fractions, (c) relative contribution of OM pools towards bulk SOC, and (d) distribution of OM pools between sites.

Note: Error bars represent standard error, and **a**lphabetic letters denote statistical differences between sites at specific depth according to the least significant difference (lsd) of means at α = 0.05. Similar letters indicate lack of significant differences.

The stable MOC content appeared relatively similar for spekboom and nonspekboom sites, *ca.* 28.0 ± 3.4 and 26.2 ± 2.9 g C kg^{-1} respectively, but significantly lower under open sites, *ca*. 17.2 ± 1.9 g C kg^{-1} (Fig. 3.3 b); comprising *ca*. 60-66 % of total organic carbon on average (Fig. 3.3 c). The residual organic carbon that was not accounted for by the POC and MOC pools was automatically considered as dissolved organic carbon (DOC), which was lost during the filtration process while collecting the POC.

The effects of grazing and resultant change in vegetation structure in the open areas has amounted to *ca*. 24 % and 39 % decline in particulate and stable organic C respectively, relative to spekboom vegetated sites. Although significant SOC losses were found in the open sites, the remaining bulk SOC content reflected here (19.5-34.0 g C kg^{-1}), of which *ca*. 66 % of it is stable, is still remarkably high compared to other semi-arid ecosystems. For instance, Kondo et al. (2010) found that the light fraction accounted for nearly 90 % of total SOC, indicating very little role of mineral sorption and SOC stabilization. Zeidler et al. (2002) reported 0.47 % (4.7 g C kg^{-1}) total SOC contents for a semi-arid savanna/woodland ecosystem in Namibia, SOC content estimated in this study is also more than six fold that estimated by Rasmussen and White (2010), i.e. 2.5 g C kg^{-1} soil, for grassy interspaces in a native desert-scrub semi-arid ecosystem. Moreover, these C content ranges are comparable to forest ecosystems; 32 g kg^{-1} under oak vegetation reported by Quideau et al. (2000), and 3.0-9.6 % i.e. 30-96 g kg^{-1} under mixed woodland vegetation by Martínez-Zavala and Jordán-López (2009) in cooler Mediterranean climates.

It is possible however that this organic C lingering phenomenon is attributable to the preceding native vegetation (spekboom), which is not uncommon for waxy e.g. Eucalyptus and most Pine vegetation types. For instance, Poirier et al. (2006) found the non-hydrolysable organic matter fraction (including the mineral-bound humin fraction) of the native pine vegetation to largely dominate the soils currently under maize cultivation, 35 years post land use change from forest to continuous intensive maize cropping system. Rietkerk et al. (1996) suggested that semi-arid savanna grazing systems can be described by state and-transition models, where the system exhibits certain feedbacks that maintain stability. The concept appears applicable in this study in the sense that the intact succulent thicket represents the initial stable state, its transition to a grassy savanna-like is illustrated by a decline in both particulate and stable organic C content, however with an invariable proportion of stable organic C compared to spekboom sites.

The OC:N ratios of the isolated fractions were consistent with other studies on organic matter decomposition e.g. Gregorich et al. (2006), Golchin et al. (1994) Sohi et al. (2001), and Grünewald et al. (2006). The OC:N ratios were greatest in the

POM fractions at all sites, although open sites displayed significantly wider OC:N ratios for POM compared to other sites (Fig. 3.4). Wider OC:N ratios signifies the dominance of plant components Gregorich et al. (2006), whereas narrowing of OC:N ratios reflects the dominance of more decomposed humified OM (Baisden et al., 2002).

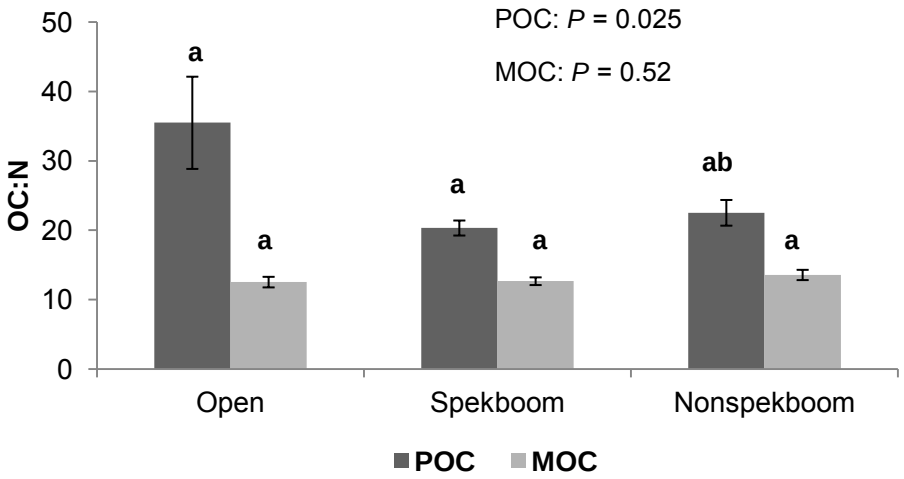

Fig. 3.4 Organic C to N ratios of free particulate OC and stable mineral-bound OC.

Note: Error bars represent standard error, and alphabetic letters denote statistical differences between sites at a specific depth, according to the Bonferroni's least significant difference (lsd) of means at α = 0.05. Similar letters indicate lack of significant differences.

Similar to some previous studies on organic carbon stability in semi-arid ecosystems (e.g. White et al., 2009); only poor relationships were found between stable organic carbon and soil properties investigated in this study. None of the hypothesized soil parameters (aggregate stability, hydrophobicity, mineralogy, and polyvalent cations) yielded meaningful correlations with stable organic carbon, as illustrated in Fig. 3.5 below. Poor correlations were between stable (mineral-bound) organic carbon and sesquioxide parameters presented here were most likely attributed to greater degree of crystallinity of minerals contained on our soils, hence marginal concentrations (<20) of active (γFe/*t*Fe Fe). This compelled further investigation into the nature of the vegetation from the investigated sites, to elucidate possible effects of vegetation chemistry on organic carbon stability.

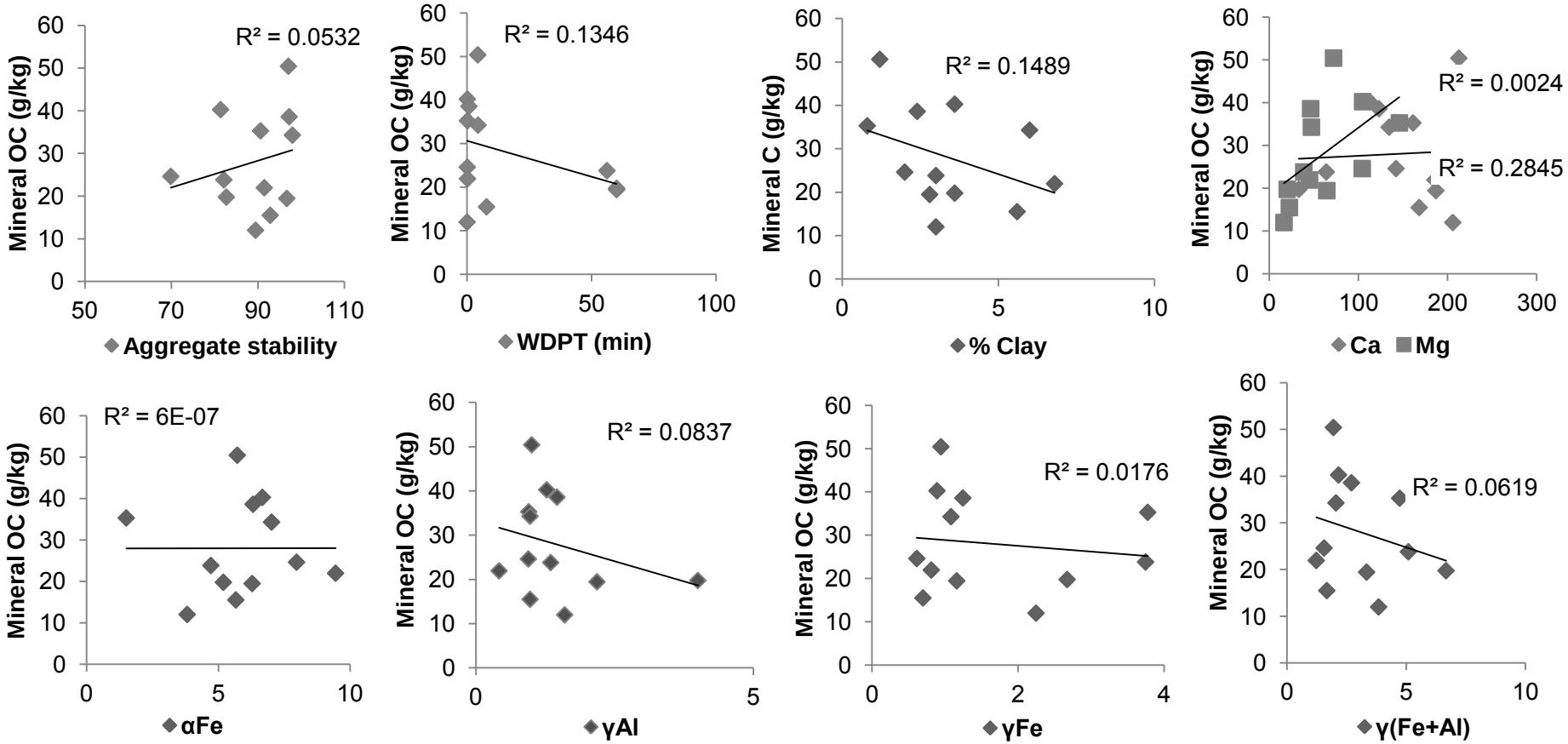

Fig. 3.5 Relationship between stable mineral-bound organic carbon and various soil parameters under spekboom.

Greater MOC content for spekboom and nonspekboom could probably be attributed to a certain degree of OM recalcitrance under the intact thicket since soil minerals have only a finite sorption capacity. The effects of leaching, erosion and UV-photo-oxidation are the most probable causal factors of the significant decrease in POC observed in the open sites, whereas, microbial decomposition represents a potential degradation mechanism pertinent to mineral-bound OM. A shift in OM substrate and consequent reduced amounts of labile, energy-rich POC, together with the prevailing micro-climatic conditions encountered in the open sites perhaps suggests lower microbial activity in the sense that these host systems (intact vs. degraded thicket) offer different habitat benefits. Furthermore, the palatability of spekboom to herbivores could imply similar appeal to other soil fauna and microorganisms further along the food-chain. These factors can be expected to impart considerable variations in diversity and community structure of soil organisms inhabiting these sites, which in turn could affect the rate of decomposition and thus resultant amounts of stable organic C, which is largely composed of microbial secretions. Hence slower POM decomposition under open sites versus accelerated decomposition under intact sites as indicated by the OC:N ratios.

3.3.4 Spectroscopic analyses

3.3.4.1 Fourier Transform Infrared (FT-IR)

Spectroscopic characteristics of litter samples were largely similar between sites irrespective of vegetation cover. Allocation of the various functional groups represented by the numbered peaks is shown on Table 3.2. The structural composition of litter was mostly dominated by aliphatic (peak 2, 3, and 7) and aromatic (peak 5) compounds, recognized as sharp adsorption peaks at a particular frequency (Fig. 3.6), carboxyl groups (peak 4 appearing as a shoulder on the left of peak 5) and polysaccharides (peaks 9-10) were also detected although not as prominently expressed. Spekboom litter appeared to have better resolved peaks of higher adsorption intensity for most functional groups, possibly suggesting greater concentrations of specific compounds relative to the other vegetation litters.

Furthermore, spectroscopic characterization of foliar material with FT-IR was mandatory in order to eliminate potential confounding effects of clustered canopies on litter inputs within the intact thicket. Foliage presented a similar spectrum pattern as that of litter samples respective to sites, although with prominently expressed peaks, especially for carboxyl and polysaccharide groups; peak 4 and 10 respectively. The main variation between spekboom and nonspekboom foliage was the relative intensities of the peaks, spekboom in particular displayed well resolved peaks, especially for aliphatic, carboxyl, and polysaccharide spectral regions (Fig. 3.7). Particulate and mineral-bound C FTIR spectra could not be interpreted due to poorly resolved, broad overlapping peaks which is one of the common drawbacks of FTIR spectroscopy.

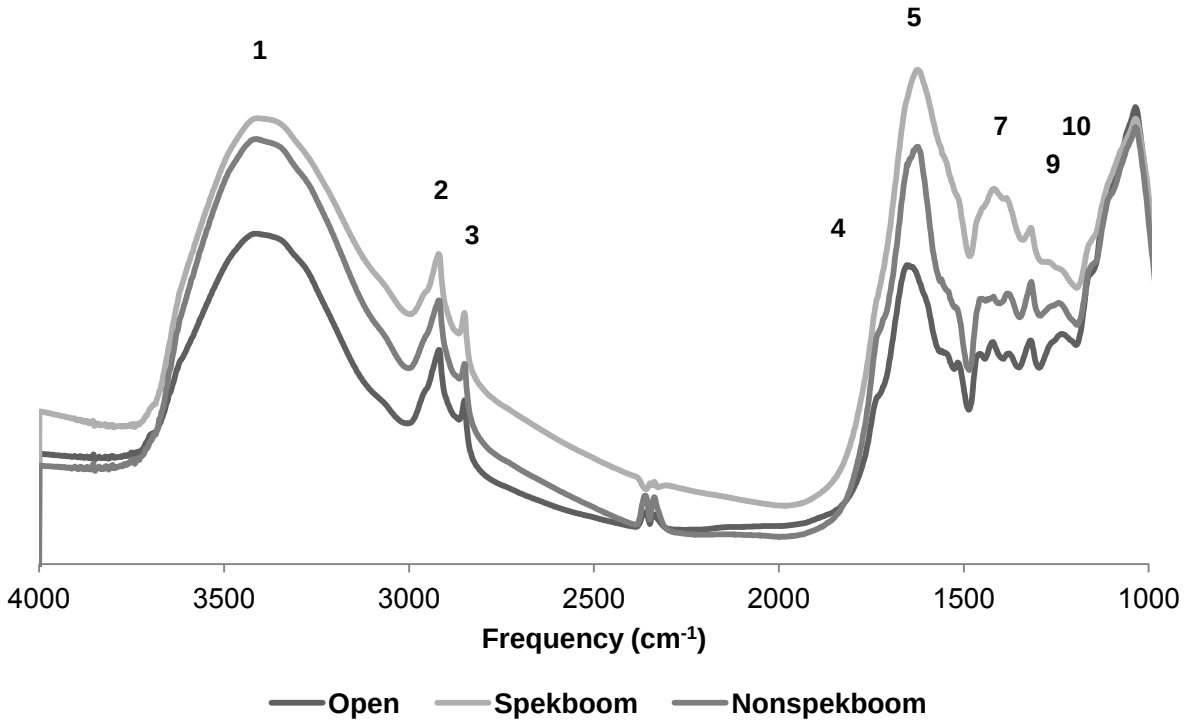

Fig. 3.6 Absorption spectra of organic litter samples from open, spekboom, and nonspekboom sites, characterized with FT-IR.

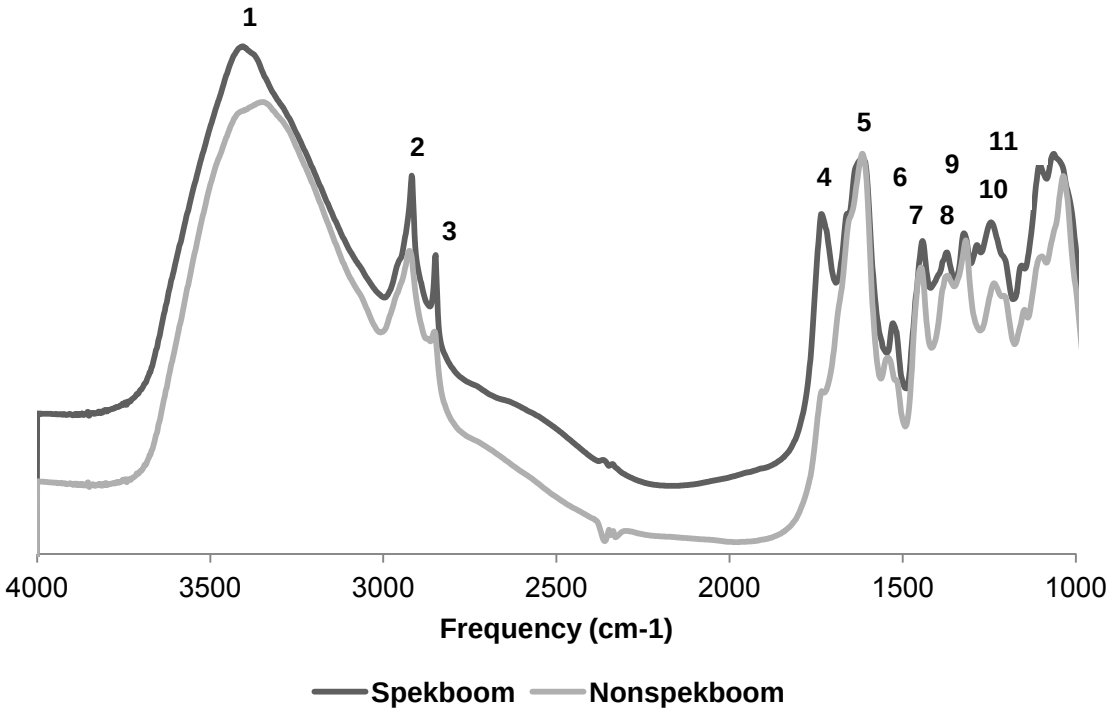

Fig. 3.7 Absorption spectra of spekboom and nonspekboom foliage (plant material) from the intact thicket, characterized with FT-IR spectroscopy.

Table 3.2 Assignment of major organic functional groups according to their characteristic adsorption frequencies, determined with FT-IR.

Peak position	Frequency (cm^{-1})	Functional groups
1	3355 - 3413	O-H (hydroxyl) stretches for water molecules
2	2915 - 2924	Substituted aliphatic C
3	2848	Unsubstituted aliphatic C
4	1727	C=O stretches of COOH
5	1612 - 1616	Aromatic C=C
6	1521 - 1535	C=C of aromatic rings
7	1440 - 1442	Aliphatic C-H
8-9	1320-1367	CH_3, COO- groups
10	1238	C-O stretches of polysaccharides and OH distortion from COOH
11	1153	Polysaccharide C-O stretches

References: Stevenson and Schnitzer (1982)

3.3.4.2 Solid state ^{13}C NMR

The spekboom litter and particulate OM was studied with solid state ^{13}C NMR, to evaluate its chemical recalcitrant nature. Spekboom vegetation was preferably selected due to its greater abundance in the thicket relative to other vegetation. The extent of decomposition can be seen in the chemistry of the particulate OM fraction (Kogel et al., 1988, Swift et al., 1979). Therefore, the quantitative chemical composition of POM was compared with that of the surface litter derived from respective spekboom vegetation using solid state ^{13}C NMR technique with CP MAS optimization. Unfortunately, the mineral-bound OC sample was not suitable for ^{13}C NMR analysis. The poor quality NMR spectrum acquired for the spekboom mineral-bound organic C was as a result of poor magnetization transfer because of the low natural abundance of ^{13}C atoms, and interference due to the presence of paramagnetic metals such as iron, which resulted in low peak intensities and great signal to noise ratio (Fig. A3.1, Appendix).

Characterization of these samples with cross polarization technique afforded a further advantage of integrating the areas beneath chemical shift ranges, in order to estimate the relative amounts of the different ^{13}C species. Chemical shift spectra acquired for spekboom litter and particulate OM were complimentary to FT-IR results with respect to organic functional group identification. Chemical shifts appeared more pronounced for the surface litter as a precursor substrate to POM (Fig. 3.8), which means that the POM is chemically more heterogeneous. Unsubstituted (*u*-) alkyl C peaks occur distinctly at 33 ppm, which is characteristic of polymethylene (straight-chain methylene) carbon species (Fig. 3.8). Substituted (*s*-)alkyl groups, were also identified with methoxyl group at 57 ppm suggesting presence of lignin. Strong resonance was observed for *u*-alkyl, O-alkyl, and aromatic C groups, with most prominent peaks at 0-45, 65-95, and 110-145 ppm, representing respectively 19.2, 21.7, and 20.2 % for litter, and 20.3, 18.9, and 22.8 % for particulate OM (Fig. 3.8 and Table 3.3). Two sets of peaks were identified within the aromatic C region; one at ca. 115 ppm and another at 120 ppm, corresponding to phenolic and unsubstituted aryl C respectively. Aromatic C species at 115 ppm often suggests that the phenolic C is most likely located within lignin structures of aromatic groups

(Golchin et al., 1994). Distinct peaks in the phenolic region (145-160 ppm) are characteristic of condensed tannins (Lemma et al., 2007).

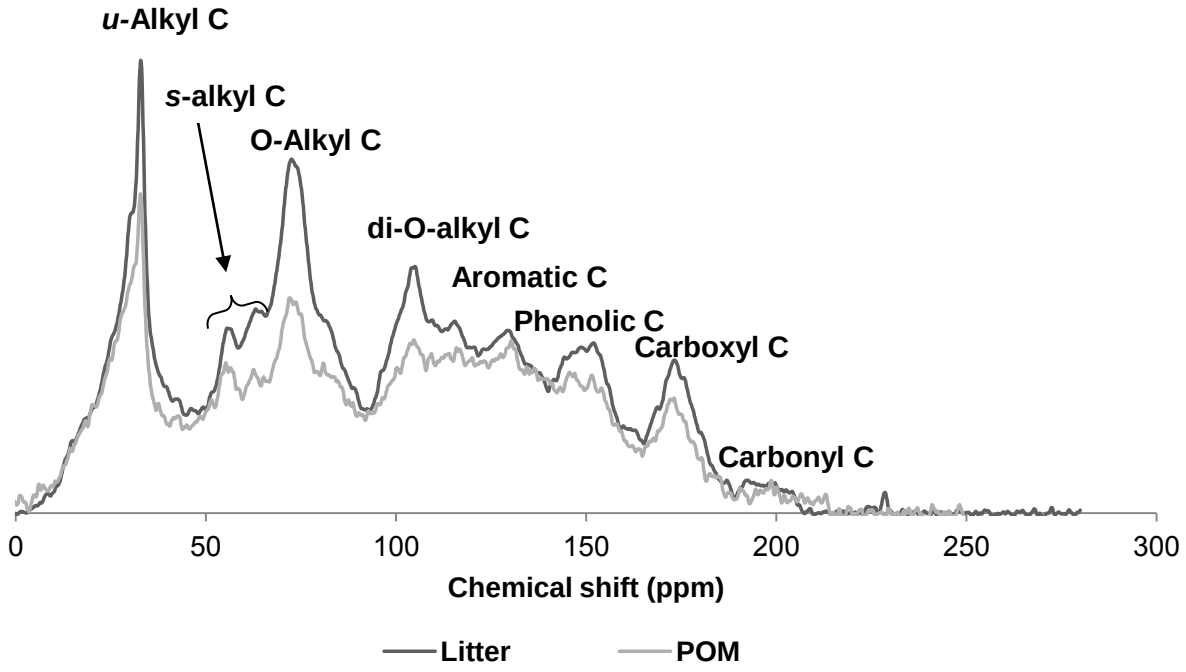

Fig. 3.8 Solid state $^{13}C^{1}H$ CP MAS spectra of the SOM from spekboom litter and particulate OM pool. *Note:* ***u*** *and* ***s*** *denote unsubstituted and substituted, respectively.*

Unsubstituted alkyl carbon forms constitutes an important structural component of natural organic matter in soils, distinction of these groups at 33 ppm is suggestive of polymethylene structures, which is mainly derived from plant waxes from cutin and suberin (Derenne and Largeau, 2001, Kögel-Knabner, 2002). The relative quantities of organic functional groups are in agreement with the successive stages of organic matter decomposition according to the decomposition index (alkyl/O-alkyl C ratio) as proposed by Baldock et al. (1992). This was manifested by a considerable reduction in O-alkyl C and a complementary increase in alkyl C from spekboom litter to particulate OM. Similar to other studies, a slight increase in the O-alkyl/alkyl ratio was noted with decomposition, from 0.88 for litter to 1.07 for POM. Marin-Spiotta et al. (2008) reported 18.0 vs. 31.2 % (0.58) and 15.7 vs. 22.4 % (0.70) alkyl to O-alkyl C for a subtropical montane forest litter and POM, respectively. This illustrates greater decomposition under spekboom which could be anticipated given the prevailing climatic conditions. Furthermore, the subtle variation in the O-alkyl C

between spekboom litter vs. POM possibly suggests slow decomposition and persistence of the POM fraction, most probably attributable to its waxy nature as suggested by the alkyl C content, also possibly due to the presence of lignin which is renowned for its high degree of recalcitrance.

Table 3.3 Quantitative integration of spekboom litter and particulate OM functional groups according to chemical shift regions.

Chemical shift (ppm)	Litter %Integral	POM %Integral	Functional group
0 – 45	19.2	20.3	Unsubstituted alkyl carbon
45 – 65	10.7	10.5	Substituted alkyl carbon (amines, methoxyl groups)
65 – 95	21.7	18.9	Oxygen-substituted carbons, ring carbons in carbohydrates, carbons in ethers
95 – 110	10.3	9.6	Di-oxygen-substituted aliphatic carbon, anomeric carbon in carbohydrates
110 – 145	20.2	22.8	Aromatic carbon
145 – 160	7.5	7.7	Phenolic carbon
160 – 190	8.9	8.4	Carboxylic, amide, ester carbon
190 – 220	1.5	1.8	Carbonyl carbon

References: Baldock and Skjemstad (2000).

Comparison of substrate composition of spekboom litter samples with other studies revealed that spekboom contains even more alkyl (lipids) groups compared to pine vegetation (*Pinus patula* and *Pinus coulteri*). Spekboom litter contained 19.2 % alkyl, 20.2 % aromatic, and 21.7 % O-alkyl C groups, compared to 16.0, 10.9, and 44.3 % found by Lemma et al. (2007) for *Pinus patula* litter in the similar spectral regions respectively. Quideau et al. (2000) reported 10.4, 16.1, and 69.0 % of total organic carbon under alkyl, aromatic, and O-alkyl regions respectively, for the *Pinus coulteri* litter.

Progressing from the litter material to POM fraction, a decrease in O-alkyl C along with an increase in alkyl (aliphatic), aromatic and carbonyl C groups was observed (Table 3.3). The O-alkyl groups comprise mainly carbohydrates, with the largest contribution from celluloses and hemicelluloses (Lemma et al., 2007), thus the observed decline is indicative of its preferential decomposition during early stages of decomposition (Quideau et al., 2000). The corresponding increase in alkyl and aromatic C suggests selective preservation or addition of new recalcitrant (alkyl-type e.g. lignin) organic materials through microbial synthesis during litter decomposition (Baldock et al., 1992). Distinct methoxyl peak at 57 ppm and well resolved peaks in the phenolic region (147 and 152 ppm) of particulate OM confirms the occurrence of lignin and condensed tannins, respectively.

It is evident that spekboom contains great quantities of recalcitrant compounds as aliphatic and aromatic C forms relative to pine vegetation. Aromatic C content of spekboom litter is closely similar to that of oak litter (20.1 %) determined by Quideau et al. (2000). Lignin and tannins are the typical contributing compounds to aromatic C (Lemma et al., 2007), given the renowned recalcitrance of lignin due to its polymeric disordered structure, slower organic matter decomposition is suggested in the intact spekboom thicket. Hence the greater organic C content obtained for whole soils and density fractions compared to other ecosystems in similar climatic regions. This emphasizes the influence of species-derived carbon chemistry on SOC content, and suggests recalcitrance as the major stabilization mechanism in the thicket. These findings validate the richness of spekboom in lipids, tannins, and cutin-like compounds, as anticipated by its succulent waxy appearance. This is possibly further enhanced by other aliphatic substances from microbial secretions.

3.4 CONCLUSIONS

Soils developed beneath woody vegetation i.e. spekboom and nonspekboom in the intact portions of the thicket seem to exhibit a greater carbon sequestrating capacity than those that have been degraded into typical savanna grasslands through unsustainable herbivore grazing. This was substantiated by a significant reduction in organic C concentrations in both particulate and mineral-bound SOM pools under open sites investigated in this study. However, appreciable amounts of organic carbon, especially stable mineral-bound C still occur in the upper 15 cm of degraded,

open landscapes, probably leading to conservation of much larger organic C than one would anticipate for a semi-arid ecosystem. Organic C content was significantly greater within the intact thicket, where animal browsing is restricted through fence boundaries. Furthermore, significant differences were found in spatial distribution of organic C concentrations between SOM pools, with respect to sites. The majority (60-66 %) of the SOC was found to be in the more stable mineral-bound fraction. The mineral-bound organic carbon was found to be on average 17.2, 28.0, and 26.2 g C kg^{-1} for open, spekboom, and nonspekboom sites, respectively. Only the open (degraded) sites were statistically different, with the least mineral-bound (stable) organic C content relative to other sites. The POM accounted for 22-34 % of total organic C, comprising *ca*. 7.0, 9.3, 14.4 g C kg^{-1} for open, spekboom, and nonspekboom sites, respectively, with each site significantly different from the other.

Despite the observed declines in SOC content, manifested in both OM fractions due to the effect of grazing animals on the open landscapes of the thicket, a substantially large proportion *ca*. two thirds of the remaining organic C is effectively stabilized in the mineral fraction. This illustrates a remarkable SOC resilience to degradation and possibly secondary stable (equilibrium) state. The organic C content reflected on open sites is probably the residual effect of spekboom vegetation from the original thicket in its native state, before land use change. Furthermore, no significant relationships were found between stable SOC and the soil chemical, physical and mineralogical parameters investigated in this study. However, carbon chemistry of spekboom vegetation pointed towards inherent recalcitrance as the second mechanism pertinent to carbon stabilization in the thicket, in addition to mineral adsorption. Recalcitrant spekboom traits are mainly attributed to the fatty (aliphatic lipids) and highly aromatic chemistry of the residue substrate.

GENERAL CONCLUSIONS AND FUTURE DIRECTIONS

It has been previously established that the spekboom thicket in the semi-arid Eastern Cape region is able to accumulate remarkably large amounts of soil carbon, and that overgrazing induced degradation of the thicket has resulted in significant soil C losses (Mills and Fey, 2004, Mills et al., 2005, Mills and Cowling, 2010). However, the partitioning of soil carbon as organic or inorganic C in intact (under spekboom, *Portulacaria afra*, and "non-spekboom" vegetation) and degraded (open) spekboom thicket has not been examined until now. Previous studies assumed that all soil C is organic in nature. In this study it was found that the majority, but not all (78-95 %) of the total C was organic, with the remaining 4-22 % identified as inorganic. Organic C content on average ranged between 19.5-34.0, 22.1-62.4, and 19.6-58.5 g kg^{-1} for open, spekboom, and nonspekboom sites, respectively. Whereas, inorganic C content on the other hand ranged between 2–9 g C kg^{-1} on average.

The most significant effects of degradation on organic C content were demonstrated in the top 25 cm of the profile. Degraded (open area) soils contained significantly lower amounts of organic C; *ca*. 34.0 ± 2.9, 26.4 ±1.9, and 21.8 ± 2.5 g C kg^{-1} at 0-5, 5-15, and 15-25 cm, in contrast to 62.4 ± 4.9, 42.1 ± 3.8, and 33.7 ± 3.3 g C kg^{-1} at corresponding depth under spekboom, respectively. The most remarkable was an approximate 46 % reduction in total soil organic C under open (34.0 g C kg^{-1}), relative to spekboom sites (62.4 g C kg^{-1}). There were no significant differences in the organic or inorganic C content between the spekboom and nonspekboom thicket sites, indicating that all the species in the thicket contribute together to C sequestration in the thicket ecosystem. However, these differences in C concentration were buffered by the soil bulk density, which minimized the degradation effects on C stocks in the degraded thicket landscapes, such that there are no significant differences in both organic and inorganic C stocks.

The chemical and physical soil properties beneath spekboom and "non-spekboom" vegetation within the intact thicket have also never been explored. Furthermore, the hydrophobic (water repellent) behavior and aggregate stability of these soils has never been acknowledged at all in the past. Results from this study indicated that soils directly beneath spekboom vegetation contained significantly higher exchangeable and water soluble Mg, Na, and K, hence pH and EC, relative to

degraded open and nonspekboom sites. This is attributed to the higher Mg, Na and K content of the spekboom litter and the higher rate of litter production by spekboom plants. Interestingly, the pH values of the nonspekboom soils were significantly lower than that of the spekboom and open soils. This could be due to the lower soil basic cation concentrations, yet high soil organic matter content. Both spekboom and nonspekboom soils were found to be more hydrophobic, and with higher aggregate stability compared to degraded thicket soils. Thus it can be concluded that the loss in soil organic carbon in the degraded thicket has significantly altered the soil structural stability and water dynamics, as hydrophobicity aids in reducing evaporative losses from soils.

Soil C stabilization is of fundamental importance in sustainable land use and natural resource conservation. The extent of organic C stability and the mechanisms accountable for its stability in soil have never been studied before in the intact and degraded spekboom thickets. The findings of the present study indicate that the largest fraction (60-66 %) of the organic C accumulating in the spekboom thicket is extensively humified and effectively stabilized through association with the mineral phase. Stable (mineral-bound) organic C however, remained indifferent within the intact thicket (28.0 vs. 26.2 g C kg^{-1} for spekboom and nonspekboom respectively), but varied significantly under open sites (17.2 g C kg^{-1}). Although the relative differences in organic C stocks between open and spekboom sites were disguised by the variation in bulk density and deemed statistically insignificant at 5 % level; the absolute differences in total C are nevertheless considerably large on a landscape scale, especially in the top 5 cm of the profile (open 136.4 ± 12.1 vs. spekboom 107.0 ± 12.3 t ha^{-1}).

Sites had a significant effect on particulate organic C distribution; 7.0 vs. 9.3, and 14.4 g C kg^{-1} for open, spekboom and nonspekboom sites, respectively. The particulate organic C comprised 22-34 % of the bulk soil organic C on average. No significant relationships were found between stable organic C and soil properties (exchangeable cations, clay content, hydrophobicity, crystalline and poorly crystalline Fe oxides) investigated in this study. This led to further investigation of the chemical structure of the vegetation foliage, litter and soil organic C using FTIR and NMR spectroscopy in an attempt to explain this remarkable SOC stability.

The structural composition and chemical speciation of the spekboom organic matter has never been characterized previously. Findings from this study indicated that spekboom litter and particulate organic C is particularly rich in aliphatic (lipids and waxes) and aromatic (typically lignin) substances; aliphatics comprised *ca*. 19.2 and 20.3 % of the litter and particulate OM, and aromatics represented *ca*. 20.2 and 22.8 % of the litter and particulate OM, respectively. These values are much higher than those previously reported in other studies for waxy vegetation such a pine litter. In soils, it is mainly these aliphatic and aromatic constituents that confer recalcitrance into soil, through aggregation and adsorption on mineral surfaces. Thus, it was concluded that inherent recalcitrance potentially plays a significant role in carbon stability in the thicket. These findings suggest a significant carbon sequestration capacity of spekboom thickets, which encourages restoration of the degraded thicket landscapes to prevent further SOC losses. Furthermore, this also substantiates that soil hydrophobicity is of vegetal origin, derived from plant waxes and lipids, and imparted into soil through litter-fall and subsequent decomposition.

There is a possibility however that part of the reflected mineral-bound organic C under open sites are remnants of residue inputs from former (spekboom) vegetation prior to transformation. Further investigation with advanced molecular techniques is recommended to identify the ^{13}C isotope signature of mineral-bound C, in order to verify its precursor vegetation. Mineral-bound (stable) C is the major determinant of organic carbon security and land use sustainability. Thus, it is recommended to evaluate associated changes pertaining to the stable SOC fraction instead of bulk C, especially for studies on terrestrial C sequestration, and evaluation of land use change effects on soil quality. The findings of this study are of fundamental significance in understanding soil carbon stabilization in semi-arid environments.

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APPENDIX

Table A2.1 Carbon distribution and OC:N ratio with depth between sites.

Site	Depth (cm)	Total C (g/kg)	Organic C (g/kg)	Inorganic C (g/kg)	OC:N
Open	0-5	35.68 ***b***	33.97 ***b***	2.18 ***a***	13.79 ***a***
	5-15	30.03 ***b***	26.38 ***b***	3.91 ***a***	15.10 ***a***
	15-25	27.24 ***a***	21.83 ***b***	5.58 ***a***	14.30 ***a***
	25-50	25.07 ***a***	19.46 ***a***	5.64 ***a***	13.48 ***a***
Spekboom	0-5	69.94 ***a***	62.41 ***a***	7.53 ***a***	16.45 ***a***
	5-15	49.26 ***a***	42.13 ***a***	7.13 ***a***	15.01 ***a***
	15-25	40.60 ***a***	33.73 ***a***	6.88 ***a***	14.72 ***a***
	25-50	26.21 ***a***	22.12 ***a***	4.30 ***a***	12.84 ***a***
Nonspekboom	0-5	66.78 ***a***	58.48 ***a***	9.13 ***a***	16.55 ***a***
	5-15	50.26 ***a***	44.54 ***a***	5.93 ***a***	15.89 ***a***
	15-25	35.60 ***a***	32.94 ***a***	3.58 ***a***	17.61 ***a***
	25-50	24.83 ***a***	19.62 ***b***	4.30 ***a***	13.79 ***a***

Bold italicized letters denote statistical differences between sites at a specific depth, according to the Bonferroni's least significant difference (lsd) of means at $\alpha = 0.05$. Similar letters indicate lack of significant differences.

Table A2.2 General soil properties with depth between the different sites.

	Open				Spekboom				Nonspekboom			
Depth (cm)	0-5	5-15	15-25	25-50	0-5	5-15	15-25	25-50	0-5	5-15	15-25	25-50
pH (H_2O)	7.68 ***a***	7.81 ***a***	7.90 ***a***	8.00 ***a***	7.72 ***a***	7.68 ***a***	7.85 ***a***	8.22 ***a***	5.8 ***b***	6.15 ***b***	6.64 ***b***	7.57 ***a***
pH (KCl)	6.69 ***a***	6.80 ***a***	6.80 ***a***	6.82 ***a***	6.63 ***a***	6.67 ***a***	6.80 ***a***	7.02 ***a***	5.01 ***b***	5.33 ***b***	5.89 ***b***	6.53 ***a***
$EC_{1:5}$ (dS/m)	0.24 ***a***	0.28 ***a***	0.35 ***a***	0.48 ***a***	0.33 ***a***	0.39 ***a***	0.36 ***a***	0.31 ***a***	0.26 ***a***	0.28 ***a***	0.27 ***a***	0.52 ***a***
S-value (mmolc/kg)	171.3 ***b***	183.1 ***b***	201.2 ***a***	203.8 ***a***	280.9 ***a***	229.2 ***a***	210.8 ***a***	221.2 ***a***	180.6 ***b***	175.2 ***b***	186.6 ***a***	194.2 ***a***
WDPT (min)	0.04 ***b***	7.20 ***a***	5.19 ***a***	1.22 ***a***	16.02 ***a***	16.14 ***a***	13.68 ***a***	0.56 ***a***	11.66 ***a***	18.96 ***a***	3.56 ***a***	0.17 ***a***

Table A2.3 Basic cation composition of plant material, litter, and mineral soil in the intact thicket.

	Open				Spekboom				Nonspekboom			
	%Ca	**%Mg**	**%Na**	**%K**	**%Ca**	**%Mg**	**%Na**	**%K**	**%Ca**	**%Mg**	**%Na**	**%K**
Depth (cm)	0-5	5-15	15-25	25-50	0-5	5-15	15-25	25-50	0-5	5-15	15-25	25-50
Foliage	n.d	n.d	n.d	n.d	**0.53 *b***	**0.74 *a***	**1.39 *a***	**1.77 *a***	**1.68 *a***	**0.48 *b***	**0.11 *b***	**0.94 *b***
Litter	**1.72 *b***	**0.19 b**	**0.053 *b***	**0.15 *b***	**3.21 *a***	**1.47 *a***	**0.39 *a***	**0.56 *a***	**3.45 *a***	**0.30 *b***	**0.074 *b***	**0.19 *b***
*e*Ca	115.65 ***a***	124.79 ***a***	129.56 ***a***	120.65 ***a***	142.91 ***a***	138.78 ***a***	137.47 ***a***	133.19 ***a***	137.69 ***a***	134.55 ***a***	136.52 ***a***	119.19 ***a***
*e*Mg	32.81 ***b***	37.60 ***a***	48.73 ***a***	55.75 ***a***	94.55 ***a***	57.94 ***a***	46.69 ***a***	54.29 ***a***	34.41 ***b***	31.52 ***a***	38.41 ***a***	50.96 ***a***
*e*Na	2.05 ***b***	2.90 ***b***	8.56 ***a***	15.97 ***a***	12.80 ***a***	9.36 ***a***	8.27 ***a***	18.64 ***a***	-1.28 ***b***	1.46 ***b***	3.34 ***a***	12.38 ***a***
*e*K	20.78 ***b***	17.77 ***a***	14.39 a***b***	11.40 ***a***	30.60 ***a***	23.14 ***a***	18.33 ***a***	15.11 ***a***	9.80 ***c***	7.67 ***b***	8.35 ***b***	11.65 ***a***
*w*Ca	2.68 ***a***	3.88 ***b***	4.46 ***a***	4.73 ***a***	4.29 ***a***	5.23 ***ab***	5.79 ***a***	4.22 ***a***	6.98 ***a***	6.66 ***a***	6.18 ***a***	4.86 ***a***
*w*Mg	1.06 ***a***	1.47 ***a***	1.69 ***a***	1.78 ***a***	3.12 ***a***	2.58 ***a***	2.40 ***a***	1.88 ***a***	3.44 ***a***	2.61 ***a***	2.55 ***a***	2.28 ***a***
*w*Na	6.08 ***b***	8.32 ***b***	12.13 ***a***	15.21 ***a***	14.11 ***a***	13.39 ***a***	12.44 ***a***	15.22 ***a***	7.37 ***b***	4.72 ***b***	6.46 ***b***	18.07 ***a***
*w*K	4.19 ***ab***	2.97 ***b***	2.13 ***b***	1.30 ***b***	6.44 ***a***	5.41 ***a***	4.63 ***a***	2.69 ***a***	3.24 ***b***	1.75 ***b***	1.59 ***b***	1.97 ***ab***

n.d. = not determined. *e* and *w* denotes exchangeable and water soluble cations in $mmol_c/kg$.

Italicized letters denote statistical differences between sites at specific depth according to the least significant difference (lsd) of means at α = 0.05. Similar letters indicate lack of significant differences.

Table A2.4 Persistence and relative frequency of soil hydrophobicity (*N*=12) and its distribution with depth.

Site	Depth (cm)	WDPT (min)	% Frequency
Open	0-5	0.04 ***b***	8
	5-15	7.20 ***a***	25
	15-25	5.19 ***a***	25
	25-50	1.22 ***a***	33
Spekboom	0-5	16.02 ***a***	75
	5-15	16.14 ***a***	58
	15-25	13.68 ***a***	67
	25-50	0.56 ***a***	17
Nonspekboom	0-5	11.66 ***a***	67
	5-15	18.96 ***a***	83
	15-25	3.57 ***a***	67
	25-50	0.17 ***a***	17

Italicized letters denote statistical differences between sites at specific depth according to the least significant difference (lsd) of means at α = 0.05. Similar letters indicate lack of significant differences.

Table A3.1 Mean OC distribution between density fractions and their contribution towards total OC of the bulk soil (*N* = 12 per site).

Pool	Site	OC g kg^{-1} soil	Stderr	OC:N	Stderr	% Fraction	Stderr
POC	Open	7.03	0.87	35.51	6.65	27.20	2.84
	Spekboom	9.29	1.03	20.34	1.08	22.72	2.18
	Nonspekboom	14.36	1.32	22.53	1.85	33.57	2.41
MOC	Open	17.24	1.38	12.54	0.76	65.93	3.17
	Spekboom	28.02	3.35	12.67	0.55	65.17	3.17
	Nonspekboom	26.20	2.91	13.57	0.74	59.69	3.94

Stderr. = standard error

Table A3.2 Crystalline and poorly crystalline Fe & Al, and OC contents of SOM pools (POC and MOC).

Transect	αFe	γFe	γFe/tFe	γAl	γ(Fe+Al)	POC	MOC
1	6.67	0.89	0.12	1.28	2.17	10.66	40.27
2	6.32	1.24	0.16	1.46	2.71	11.64	38.60
3	5.72	0.94	0.14	1.01	1.95	10.49	50.61
4	3.82	2.24	0.37	1.60	3.85	6.60	12.01
5	6.28	1.16	0.16	2.18	3.34	11.37	19.46
6	5.66	0.70	0.11	0.98	1.67	10.02	15.51
7	1.51	3.77	0.71	0.95	4.73	5.59	35.29
8	5.19	2.67	0.34	4.00	6.67	4.24	19.77
9	4.72	3.74	0.44	1.35	5.09	5.30	23.82
10	7.02	1.08	0.13	0.98	2.06	14.20	34.29
11	7.97	0.61	0.07	0.95	1.56	14.84	24.62
12	9.45	0.81	0.08	0.42	1.23	6.50	21.96

All values reported in g kg^{-1}, α, γ, and *t* denote crystalline, poorly crystalline, and total amounts of Fe or Al, respectively.

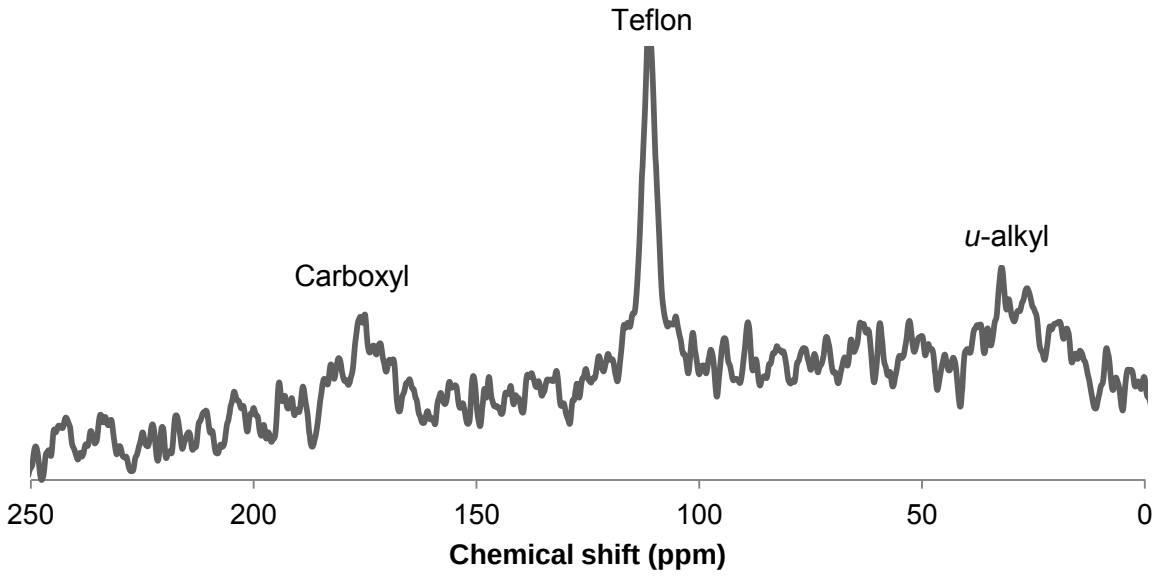

Fig. A3.1 Solid state CP MAS ^{13}C NMR spectra of mineral-bound organic C. (Note: *peak labeled "Teflon" refers to sample-holder material*)